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Observations on the Biology of *Fomes annosus*, with Particular Reference to East Anglian Pine Plantations

II. Spore Production, Stump Infection, and Saprophytic Activity in Stumps

BY

J. RISHBETH

(*Botany School, University of Cambridge*)[1]

With Plate I

SUMMARY

F. annosus produces conidia abundantly in culture but rarely under natural conditions. Sporophores are formed on stumps and dying trees throughout the year provided that the humidity is sufficiently high. Spore discharge in the plantations may occur at any time of year, but slows down or ceases during very dry weather. It takes place at low temperatures, ceasing only when sporophores freeze.

In the laboratory, *F. annosus* basidiospores germinate in relative humidity exceeding 92 per cent. on freshly cut, unsterilized pine wood. Viability is lost rapidly in light at temperatures above 15° C.

Under humid conditions *F. annosus* spores will infect freshly cut pine stumps.

There is evidence that spores of *F. annosus* washed down into soil remain viable for at least a short period and that stumps covered with soil are infected from this source. *F. annosus* spores can infect stumps for only a few weeks after felling, colonization after a longer interval probably being prevented by competing fungi. In stumps inoculated with a mixed suspension of *F. annosus* and *Peniophora gigantea* spores, *Fomes* at first colonized the wood but was soon replaced by *P. gigantea*. Other aspects of competition are discussed.

The incidence of natural infection in stumps and the factors affecting it are discussed.

Growth rates of *F. annosus* on malt agar and in lengths of pine root are given. The fungus grows in stump roots at about 1 m. per year, and so closely approaches adjacent trees within a year of thinning the plantation. In large stumps, *F. annosus* may survive 15 or even 30 years after felling. Data are given which suggest that the infective capacity of stumps containing *F. annosus* is greater in alkaline than in acid soils, while replacement by other fungi is slower.

Competition of *F. annosus* with other fungi during colonization of stumps is discussed. The parasite can grow along roots already occupied by certain fungi but not along roots containing others. The probable course of succession in stumps rotted by *F. annosus* is described.

SPORES OF *F. ANNOSUS*

i. *Production*

FOMES ANNOSUS is somewhat unusual amongst wood-rotting basidiomycetes in that it produces conidia in addition to basidiospores. Conidia, although formed abundantly on agar and other media in the laboratory, are

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rarely found under natural conditions. They have been seen in East Anglia around the entrance of holes made by bark-beetles at the sheltered base of young pines infected with *F. annosus*. They have also been seen under the bark of a dead pine, just above ground level. When cultures were made on freshly cut wood surfaces, suspended above salt solutions in van Tieghem cells, conidia were not formed below a relative humidity of 95 per cent., although slow mycelial growth took place at slightly lower humidities. Conidia were formed from 0° C. to 22·5° C. and in light or darkness. Cultures sealed with paraffin wax for several weeks in the laboratory failed to develop conidia during growth, although conditions were otherwise satisfactory. This suggests that oxygen concentration is a limiting factor, and may explain the absence of conidia on infected roots below ground even where there is space for their development. It is unlikely that conidia play an important role in dispersal.

Sporophores are produced throughout the year on stumps and dying trees (Pl. I, A and B) provided that the humidity is sufficiently high. During dry weather in summer, for instance, sporophores are often present in unbrashed plantations but not in brashed ones, which allow a freer circulation of air (brashing entails the removal of lateral branches to a height of about 2 metres). Similarly, sporophores are often found on stumps covered by a pile of branches or in deep stump cavites when they are absent on relatively exposed stumps. The most usual position for sporophores to develop is at the soil surface or a few centimetres above; in a sheltered situation, however, one was seen 45 cm. above the ground. Sporophores are formed in tunnels made by small mammals, where these pass close to diseased roots. Lastly, they are found in the litter of some plantations, attached to very fine rotted roots (Pl. I, C and D), sometimes several metres distant from the dead tree to which the roots belong.

Bjørnkær (1938), working in Denmark, found that sporophores of *F. annosus* discharge basidiospores continuously except during severe frost. Sporophores gave spore-deposits in the laboratory when gathered from East Anglian plantations towards the end of the severe drought in the summer of 1947 and also during exceptionally cold weather earlier the same year. Since a small spore deposit was obtained at 0° C., spore production under natural conditions probably does not cease until sporophores freeze.

A few observations were on spore discharge from sporophores in the plantations. A malt agar plate, clamped to a small retort stand, was exposed within a few centimetres of the spore surface and down-wind from it. A similar plate, exposed a few metres away upwind, acted as control. Two successive exposures, each of 10 minutes, were made in each position. The plates were incubated at 22·5° C. and the number of *F. annosus* colonies which developed were counted. If colonies grew densely, they were sub-cultured after 2–3 days to obtain a more satisfactory count. The counts doubtless underestimated the number of spores liberated but nevertheless provide interesting data. Observations were made at monthly intervals during 1948 on a small sporophore which appeared at the base of a dead tree late in January. From

May to December an average of 10 *F. annosus* colonies developed on each plate exposed near the sporophore, although the number rose to 20 in September. Single colonies occasionally appeared on the control plates, and presumably arose from spores liberated by neighbouring sporophores. By the end of the year the sporophore was dead, but meanwhile another had developed adjacent to it. This discharged spores during January and February 1949, ceased doing so in March, and in turn died. The weather was cold early in the winter and in November spores were discharged when the air temperature was only 3° C. The second sporophore liberated spores abundantly after a spell of much colder weather during which, according to local records, minimum air temperatures ranged from –3° to –7° C. These observations suggest that, in East Anglia, spore production is not greatly restricted by low temperatures.

Rainfall data are of interest in this connexion. Monthly totals in February and March 1948 were only 0·8 and 0·7 in. respectively, which may well have retarded development of the first sporophore. While it was discharging spores, from May to December, the monthly rainfall totals did not fall below 1·3 in. and often exceeded 2·0 in. Only 1·0 in. rain fell during the month following the first observation on the second sporophore (mid-January 1949) and in the succeeding 3 weeks as little as 0·2 in. fell. This unusually dry spell in late winter probably accounts for the early cessation of spore discharge. Early in March the same year, agar plates were exposed near eight sporophores situated on stumps: spore production was recorded in three instances only, and even so it was at a very slow rate. By contrast, a similar observation made in late October 1949, after a month in which 3·7 in. rain fell, suggested that the rate of spore discharge was some 50 times greater than in March. It seems likely, therefore, that spore production slows down or even ceases during spells of very dry weather.

ii. *Germination and viability*

Some conditions under which spores germinate were investigated in the laboratory. To determine the relation of humidity to germination, sections of pine stem about 5 mm. square and 1 mm. thick were cut under aseptic conditions and attached to sterile coverslips by means of molten paraffin wax. The coverslips and sections were exposed beneath a fresh, clean sporophore of *F. annosus* to obtain a spore deposit and were then fixed in van Tieghem cells above salt solutions giving various relative humidities. The cells were kept at various temperatures and examined under the microscope at intervals. The results obtained after keeping sections for 9 weeks at various humidities are given in Table I; for each test performed, '+' indicates that *F. annosus* mycelium appeared and '—' that it did not appear.

In another experiment with wood sections kept at 20° C., mycelial growth was obtained at 92·6 per cent. R.H. but not at lower humidities. From these results it appears unlikely that basidiospores would germinate on stump surfaces below a relative humidity of about 92 per cent.

TABLE I

The Relation of Humidity to Germination of F. annosus *Basidiospores on Unsterilized Pine Wood.*

Storage temp. (° C.)	% relative humidity given by: Distilled water.	Solutions of sodium chloride.			
	100	97·5	95·2	92·6	90·2
5	+	+, +	+, +	−, −	−
10	+	+, +	+, +	−, −	−
15	+	+, +	+, +	−, −	−

The period of viability of basidiospores on wood sections under various conditions was also determined. Wood sections bearing a spore deposit were kept in cells at various humidities as already described, or were placed in loosely plugged, sterile glass tubes. The cells were stored in rooms where temperature was more or less constant and the tubes in an unheated greenhouse or outdoor shelter, where temperature and humidity were recorded by thermohygrograph. Sections were transferred at intervals to a saturated atmosphere and production of typical *F. annosus* conidia under these conditions was used as a test of viability. The results are given in Table II.

TABLE II

The Approximate Period of Viability of F. annosus *Basidiospores on Wood Sections kept under Various Conditions.*

Temperature (° C.)	Humidity (% R.H.)	Illumination.	Period of viability (weeks).
A. Conditions constant			
15	93	−	26
15	80	−	28
15	58	−	17
15	32	−	40
20	66	−	6
20	66	+	1
B. Conditions fluctuating			
5–20 (11)	30–85	+	6
9–21 (16)	20–90	+	<1
10–35 (18)	30–90	+	<3
10–30 (19)	30–90	+	<1
7–33 (20)	30–90	−	11

Notes. Temperature: in B, the approximate mean is given in brackets.
Illumination: '+' denotes storage in light and '−' storage in darkness.

For wood sections kept at 15° C., humidity had little effect on the period of viability. Viability was maintained longest with sections kept in darkness at 15° C., whereas it was lost soonest with sections kept in light at temperatures above 15° C. Of the two factors, light possibly had the greater influence.

Basidiospores of *F. annosus* which settle on stumps in plantations probably remain viable during short spells of cool dry weather. During warm dry weather spores are unlikely to remain viable more than a few days, especially on stumps exposed to full sunlight.

STUMP INFECTION

i. *Experimental infection of stumps by spores*

Great interest is attached to the question of stump infection by air-borne spores. Petch (1921) laid emphasis on the colonization of jungle stumps in the tropics by this means; he believed that dangerous parasites such as *Fomes lignosus*, *F. noxius*, and *Ustulina zonata* entered stumps and then emerged from stump roots to infect the roots of young plantation crops. No experiments to prove this hypothesis were reported then, nor have they been since. Subsequently some circumstantial evidence conflicting with this idea accumulated (Garrett, 1944). Leach (1939) completely failed to obtain stump infection with basidiospores of *Armillaria mellea*. Napper (1934) considered that all root disease inoculum giving trouble in young plantation crops of rubber was present on the roots of original jungle trees. His methods for control of root disease, based on this alternative suggestion, proved successful, and thus considerable doubt arose about the importance of stump infection by spores. Although the position may differ in temperate climates, it is important to discover whether parasites can infect stumps in this way. If it is true, as suggested in an earlier paper (Rishbeth, 1950), that *F. annosus* is initially absent from plantations on former heath and arable, stumps left by thinning should provide an opportunity to determine whether the fungus is established by spores.

In a preliminary experiment, fifty 4-year-old Scots pines, which had been potted in Breckland soil,were cut just above soil-level. The cut surface was immediately inoculated with a suspension of basidiospores and then covered by a moist, sterile test-tube. The tubes were removed after a week, when the cut surfaces were covered with soft grafting wax to exclude wind-borne spores. The soil was kept moist by watering until the experiment was complete. Seven months later, 20 of the 50 small stumps contained *F. annosus*, which in some instances had grown 15 cm. or more from the cut surface into the branching roots.

To obtain evidence under more natural conditions, further experiments were carried out in a plantation of 20-year-old Scots pines. Relatively small trees, of diameter 6–8 cm. at the base, were chosen since it was proposed to cover a proportion of the stumps with tins only slightly larger than this. Sixty such trees were felled 10–15 cm. above soil-level. Twenty stumps were sprayed at once with a suspension of *F. annosus* basidiospores and covered by sterile tins. If the surface soil was dry at the time, some of it was moistened and packed around the base of each tin. Twenty other stumps were sprayed with a spore suspension but left uncovered, and the third lot of 20 were not treated in any way. A month later the stumps were sawn off at soil-level and brought back to the laboratory. They were quartered, wrapped in moist paper, and incubated for 7 days at room temperature. The number of samples producing characteristic conidiophores of *F. annosus*, and hence containing the fungus, was then determined. Results obtained from stumps in two plots, cut respectively in July and August 1948, are given in Table III.

TABLE III

Colonization of Pine Stumps by F. annosus *Basidiospores: the Number (out of 20) containing the Fungus One Month after Inoculation*

	Treatment of stumps:		
	Inoculated covered.	Inoculated not covered.	Untreated.
Plot 1 . . .	17	18	1
Plot 2 . . .	16	14	0

The extent of natural infection in each plot, as shown by the percentage of untreated stumps containing the fungus, was small by comparison with that of artificial infection. It is somewhat surprising to find that *F. annosus* colonized uncovered stumps as readily as covered ones, above which a moist atmosphere was maintained. Particularly is this so with plot 1, where inoculation was followed by a fortnight of very hot, dry weather. When stumps are inoculated in the manner described, however, some spores are carried down into the tracheides as far as 0·5 cm. below the surface. Since spores may germinate within 24 hours of incubation, stumps are unlikely to dry out rapidly enough to check germination. This result agrees with that of Brooks and Moore (1923), who showed that basidiospores of *Stereum purpureum* are carried into exposed vessels of plum branches.

In another experiment a high incidence of infection was obtained in uncovered stumps which had been inoculated with a suspension of *F. annosus* conidia.

Since it was not clear whether spores must be washed down into the tracheides before stump infection occurs, basidiospores were applied to stumps in a dry medium instead of in suspension. For this, dry sawdust was passed through a 1-mm. sieve and autoclaved in Petri dishes. The dishes of sawdust were then exposed beneath sporophores of *F. annosus* to obtain a spore deposit. When trees of the type already described were felled, the stump surface was sprinkled with well-mixed sawdust inoculum. Stumps were left for a month and then sampled as before. In one experiment six stumps inoculated in this way were covered by tins. Five of these were colonized by *F. annosus*, and hence the maintenance of an almost saturated atmosphere is alone sufficient to cause infection when spores are present on the stump surface. Of twelve similar uncovered stumps, wetted by heavy rain soon after inoculation, nine were found to contain *F. annosus*. Of twenty uninoculated stumps cut at the same time, none were colonized by the fungus. When *F. annosus* spores are present on freshly cut stumps, therefore, normal exposure to wet weather may lead to a high proportion of infections. Nine additional stumps were inoculated with sawdust and covered by tins, supported so that the stump surface was protected from rain although air could circulate freely around it. After a month, *F. annosus* was found in all these stumps. Records obtained from a thermohygrograph situated in the same plantation showed that, shortly after this experiment was set up, the relative humidity remained above 90 per cent. for several days. When humidity is sufficiently high, therefore, *F. annosus* will colonize stumps even if spores are not washed down into tracheides.

In another experiment, freshly cut stumps of 7-year-old pines were inoculated with sawdust containing spores. Twenty such stumps were wetted by dropping sterile water on to the surface in order to simulate rainfall and twenty others were left dry; neither set was covered. After inoculation there were four warm dry days, followed by showery weather. A month later seven of the first set of stumps but none of the second set contained *F. annosus*. Some of the first set of stumps may well have been infected from spores washed down into the tracheides. Spores on the second set probably lost viability before rain came (cf. Table II), for the experimental site was very exposed.

These investigations show that *F. annosus* spores can infect freshly exposed pine stumps. Colonization takes place readily if spores are washed into the tracheides but may also occur if a high humidity is maintained for a period at the stump surface. During dry weather *F. annosus* may fail to colonize stumps even if its spores are present on the cut surface, since their period of viability is limited.

ii. *Evidence that stumps are naturally infected by spores*

Basidiospores of *F. annosus* are not only capable of infecting stumps but are produced in sufficient abundance to do so throughout the plantations. Evidence for believing that recently exposed stumps are infected in this way will now be considered.

F. annosus becomes established in plantations on former heath and arable, from which it is initially absent, as a result of thinning the trees. The fungus is sometimes found in stumps within a month of this operation. When such stumps were incubated it was often possible to find colonies of *F. annosus* scattered over the cut surface; the mycelium extended downwards only a centimetre or so into the wood. In naturally infected stumps 2 months after felling, the fungus is present in thin wedges of wood extending downwards from the cut surface to a region just below soil-level; it is not present in the bark. In still later stages *F. annosus* colonizes the wood extensively and regularly grows on to the inner surface of the bark. Small brown and white pustules on the stump surface often indicate the presence of the fungus before sporophores are formed. *F. annosus* can always be found growing along lateral roots in a direction away from the stump, which again indicates that the latter is infected first. These observations are consistent with infection of the cut surface by air-borne spores.

Before further evidence is considered, the sampling method used for larger stumps (diameter > 15 cm.), as opposed to that already given for smaller ones, will be described. Bark samples were preferred to wood samples, being far simpler to collect. Because *F. annosus* does not appear on the inner surface of the bark until a year or so after felling, however, stump sampling was correspondingly delayed. The reliability of the method was checked by taking bark and wood samples from 40 stumps. The two sets of samples agreed with respect to the presence of *F. annosus*. In each plot or compartment where the incidence of infection was to be determined, 20–30 of the larger stumps were selected. If a small sporophore of *F. annosus* was found, the presence of the fungus was recorded and no sample was taken. If no sporophore was present, the bark was forced away from the wood around the whole stump down to the origin of the main lateral roots. Some stumps had no mycelium visible on the inner surface of the bark or had mycelium which was clearly not that of *F. annosus*; such stumps also were not sampled. Bark samples were taken, however, from stumps having mycelium like that of *F. annosus*, and these samples were wrapped in moist paper and incubated for a week. Those which produced typical conidia must have come from stumps containing *F. annosus*. Since the total number of stumps examined was known, the percentage containing *F. annosus* could be calculated. An application of this sampling method is given below.

In one experiment stumps were treated in various ways immediately after felling. A plantation of 21-year-old pines on former arable was divided up into plots one chain wide. Immediately after thinning, the surfaces of stumps in some of these plots were creosoted, painted, or covered with 4–5 cm. of soil; in other plots they remained exposed as in normal practice. These plots, each containing stumps treated in one way, were repeated along the length of the plantation. A year after felling, samples were taken and the proportion of stumps containing *F. annosus* calculated. The results are given in Table IV.

TABLE IV

The Percentage of Stumps infected by F. annosus *following Various Treatments of the Cut Surface carried out immediately after Felling.*

Treatment.	No. of plots.	Total No. of stumps examined.	Mean % stumps containing *F. annosus*.
Creosoted	4	213	4
Painted	4	211	4
Covered with soil	3	112	51
Left exposed	4	228	36

The incidence of infection by *F. annosus* was much lower in creosoted or painted stumps than in those which were left exposed. This result supports the hypothesis that *F. annosus* colonizes cut surfaces by air-borne spores, since, if the fungus always entered from the soil or roots, no measure of control by this method would be expected. The fact that *F. annosus* appeared in a few of the creosoted or painted stumps may have been due to faulty covering or to development of cracks in the wood as the stumps dried out.

The high incidence of infection in stumps covered with soil was unexpected, since it was thought that other fungi would colonize them. It was not clear how these stumps became infected and therefore further experiments were performed. Stumps which, immediately after cutting, were covered with soil and then by a tin were colonized by *F. annosus*, and therefore infection does not depend on spores being washed down through the soil. An attempt was then made to discover whether sand initially free from *F. annosus* would infect stumps after a period of exposure in the plantations. Moist quarry sand, obtained from a site well outside the forest area, was exposed during May–July 1949, in glass jars and earthenware pots in several plantations. The jars and pots were partially sunk in the soil to limit evaporation, but their rims were sufficiently high above it to escape splashing. The pot bases rested on flints in order to prevent contact with the underlying soil. Similar pots of sand, acting as controls, were covered with cellophane and an inverted pot saucer. After the interval stated the sand was used to cover freshly cut stumps, care being taken to prevent drying out and contamination by air-borne spores. Each stump surface was covered with well-mixed sand from one jar or pot. Other stumps were covered with similar sand brought straight from the laboratory. After six weeks, stumps were sampled and the number containing *F. annosus* determined. The results are given in Table V.

TABLE V

Infection with F. annosus *obtained by covering Freshly Cut Stump Surfaces with Quarry Sand treated in Various Ways*

Initial treatment of sand.	Results of covering stumps with the sand: No. (out of 20) containing *F. annosus*.
In pots, exposed	12
In pots, covered	0
In jars, exposed (plantation 1) . .	18
In jars, exposed (plantation 2) . .	19
None	1

F. annosus sporophores were moderately frequent in the plantation where sand was exposed in pots and abundant in the plantations where it was exposed in jars. The sand became highly infective after exposure in these plantations, almost certainly by the arrival of air-borne spores, since sand in the covered pots did not become infective.

Observations on the effective capacity of plantation soil are also interesting. Surface soil obtained from one site infected about 50 per cent. of the stumps covered with it in November 1948 and again in February and August 1949. It infected no stumps in May 1949. A possible explanation is that *F. annosus* spores in the soil are viable for a limited period only and that few or no viable spores were present in May, following unusually dry weather in January to March. In February 1939 soil obtained from a depth of 50–90 cm. in the same site infected 20 per cent. of the stumps covered with it, which suggests that some spores were washed deep into the soil.

The proportion of stumps naturally infected with *F. annosus* is related to their size. In one plantation stumps of the same age (about 3 years after felling) were divided into three groups on a basis of size. In each group 30 stumps were sampled and the proportion containing *F. annosus* determined. The results are given in Table VI.

TABLE VI

Natural Infection of Stumps of the Same Age: the Proportion containing F. annosus *in Relation to Size*

Diameter of stumps (cm.)	5–7	8–13	14–20
Approximate mean area of cut surface (sq. cm.) .	29·2	91·7	234·0
% containing *F. annosus*	6·7	16·7	46·7

Although relatively few stumps were examined, the figures suggest that the frequency with which stumps contained *F. annosus* was related to their surface area. If stumps are infected by spores, the chance of infection would be expected to increase with the area of exposed wood. The fact that such a relationship exists further supports the hypothesis that stumps are infected in this way.

iii. *Further data on stump infection by spores*

The ability of *F. annosus* to infect stumps at various intervals after felling was determined. After many trees had been felled, 20 stumps were left as controls, and another 20 were inoculated at once with a suspension of basidiospores. Further lots of 20 stumps were similarly inoculated at weekly intervals after felling. Control stumps were sampled a month after felling and the others after inoculation. The number of stumps in the various lots which contained *F. annosus* is given in Table VII.

TABLE VII

The Number of Stumps (out of 20) infected by F. annosus *following Inoculation with Spores at Various Intervals after Felling*

	At once.	Inoculated after an interval (weeks).				Not inoculated (natural infections).
		1	2	3	4	
Expt. 1 . . .	17	11	5	—	—	2
Expt. 2 . . .	20	17	13	9	8	7

In each experiment the ability of *F. annosus* to infect stumps decreased markedly as the interval between felling and inoculation increased. In the second experiment, with an interval of 3 or 4 weeks between felling and inoculation, stumps thus infected did not greatly exceed in number those naturally infected. These results suggest that spores of *F. annosus* can infect stumps for only a limited period after felling. Since many other fungi occur in such stumps, it is likely that colonization by *F. annosus* is chiefly prevented by increasing fungal competition. Brooks and Moore (1926) have similarly explained their observation that *Stereum purpureum* infects exposed plum branches for only a limited period after cutting.

In another experiment, freshly cut stumps were inoculated with a spore suspension of *F. annosus* and with mixed suspensions containing spores of *F. annosus* and other basidiomycetes. *Stereum sanguinolentum* (A. & S.) Fr., *Polystictus abietinus* (Dicks.) Fr., and *Peniophora gigantea* (Fr.) Massee were chosen since they were very common on pine stumps. The mixed suspensions contained roughly equivalent numbers of viable spores of *F. annosus* and one of the other fungi, as indicated by plating on malt agar. Stumps were inoculated in groups of 20, half of which were sampled after 1 month and half after 3 months. The numbers which contained *F. annosus* after various treatments are given in Table VIII.

TABLE VIII

The Number of Stumps (out of 10) which contained F. annosus *after Inoculation with Various Spore Suspensions*

Interval after inoculation (months).	Suspensions.*				Not inoculated (natural infections).
	1	2	3	4	
1	10	9	10	10	1
3	4	4	3	0	0

*1. *F. annosus* only
2. ,, + *Stereum sanguinolentum*
3. ,, + *Polystictus abietinus*
4. ,, + *Peniophora gigantea*

After 1 month nearly all the inoculated stumps contained *F. annosus*. After 3 months *Peniophora gigantea* was generally abundant in all groups of stumps as a result of natural infections. Its growth was sufficiently vigorous in stumps originally inoculated with suspensions containing spores of *F. annosus* and *P. gigantea* to replace the former entirely. The survival of *F. annosus* in stumps inoculated with suspensions containing *Stereum sanguinolentum* and *Polystictus abietinus* suggest that these fungi are less likely to replace *F. annosus* than is *P. gigantea*, which supports results given in an earlier paper (Rishbeth, 1950). The chief point of interest is the relatively short survival of *F. annosus* in stumps inoculated simultaneously with spores of *F. annosus* and *P. gigantea*. It follows that the absence of *F. annosus* in un-inoculated stumps, sampled a year or so after felling, does not necessarily indicate that the fungus failed to colonize them initially.

Experiments were also carried out to determine whether the incidence of natural infection of stumps varied at different times of the year as a result, for instance, of variation in the condition of the stumps or in the amount of fungal competition. A number of plantations on former arable land were chosen for this purpose: the trees were 15–19 years old and had not previously been thinned. Each plantation was divided into 12 plots, and these were thinned during successive months, starting in January. Such thinning was carried out in 6 plantations during 1946 and in 3 others during 1948. A year to eighteen months after thinning 20–30 stumps in each plot were sampled; the results obtained are given in Table IX.

TABLE IX

The Percentage of Stumps infected by F. annosus *in Plots thinned during Successive Months in 1946 (Six Plantations) and 1948 (Three Plantations)*

1946 Plantation No.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1	88	74	50	56	9	7	30	54	26	15	24	16
2	—	32	33	19	7	3	9	24	25	18	3	—
3	32	29	40	19	15	11	15	10	25	32	6	4
4	34	37	41	23	3	10	3	3	9	6	5	10
5	22	33	35	33	12	3	0	0	17	10	8	0
6	41	55	59	31	15	19	13	23	24	33	17	10
Average*	43	43	43	30	10	9	12	19	21	19	11	8
1948 Plantation No.												
1	16	52	24	0	0	0	0	8	28	0	0	—
2	—	0	0	5	4	0	0	0	9	23	13	18
3	8	8	8	0	4	0	0	8	8	8	0	0
Average*	12	20	11	2	3	0	0	5	15	10	4	9

* Average % stumps infected in the monthly plots.

From the previous history of these plantations it is unlikely that *F. annosus* was widespread before the experiment started, and it is therefore probable that most if not all of the infection recorded arose from air-borne spores. The varying incidence of infection throughout the year is of considerable interest, but the reason for it is obscure.

Some stumps produced such a copious flow of resin that the surface was sealed and fungi were still absent at the time of sampling. Such stumps were rather more abundant in spring and early summer than at other times of the year and therefore the proportion of stumps infected by *F. annosus* over this period was slightly lower. No other variation in the condition of stumps was observed. Records near the plantation suggest no clear relation between infection of stumps by *F. annosus* and the weather before or after thinning. The tendency for stump infection to be slight in May and June and to increase in late summer or autumn possibly arises from differences in spore discharge, such as those already described. Exact correspondence between the amount of spore discharge and stump infection by *F. annosus*, as indicated by sampling a year or so after felling, is unlikely because of competition by fungi such as *Peniophora gigantea*. Indeed, the number of *F. annosus* spores in the air may well influence the extent of stump infection less than the balance between such spores and those of competing fungi.

Although stump infection shows certain general trends throughout the year, if differs considerably from one plantation to another. For instance, more stumps were infected with *F. annosus* in plantation 1 (1948) during January to March than in other plantations thinned at the same time. This may have arisen from the presence of *F. annosus* sporophores along the northern and eastern margins of plantation 1, since prevailing winds during the period mentioned would have carried spores towards the exposed stumps. The suggestion that local variation in abundance of *F. annosus* sporophores influences stump infection agrees with the conclusion of Gregory (1945) that the chance of infection falls off rapidly with increasing distance from the source of spores.

Stump infection by *F. annosus* during abnormally dry weather in July to September 1947 was also investigated. Of 44 plots thinned during this period, 16 had more than 60 per cent. of the stumps infected by *F. annosus*. By contrast, of 124 plots thinned throught 1946 and 1948, when there was no comparable drought, only 2 had a stump infection as great as this. These observations possibly indicate that prolonged dry weather curtails spore production of *F. annosus* less than that of its competitors since, if spores of competing fungi are very few or absent, even a low rate of spore discharge by *F. annosus* could lead to extensive stump infection. Observations suggest that sporophores of *F. annosus*, because of their structure and frequent development in cavities, withstand drought more effectively than those of *Peniophora gigantea*, *Polystictus abietinus*, and *Stereum sanguinolentum*.

During the investigation it was found that, in two sites out of three replanted after fire, young trees were being killed by *F. annosus*. In the site without disease, the first crop had been planted only 3 years before the fire and no stump remains could be found. In the other two, the previous crop was 13 years old at the time of the fire; the stumps were easily traced and many contained *F. annosus*. In the laboratory, *F. annosus* basidiospores germinate on freshly charred surfaces of pine stem and the fungus then colonizes unaffected tissues beneath. It seems probable therefore that charred stumps are also infected by air-borne spores, the chance of establishment possibly being increased by destruction of fungal competitors in the bark.

It is probable that *F. annosus* can colonize stumps of trees broken by wind, but no instances of this has been seen. On the other hand, the fungus is unlikely to colonize stumps of uprooted trees, which are subjected to drying out, since it requires a relatively high moisture content for growth in wood (Bavendamm and Reichelt, 1938). In one plantation many stumps of trees cut abnormally high (average 1·2 m. from the ground) were infected by *F. annosus*, so that a high stump does not necessarily prevent entry of the fungus. Stumps of trees broken by wind or destroyed by fire might conceivably act as centres of infection in virgin coniferous forests.

iv. *Possible alternative methods of stump infection*

Transfer of *F. annosus* from one stump to another by wood-boring insects such as *Hylastes ater* Payk. is theoretically possible in view of their feeding habits. However, when beetles feeding on wood containing *F. annosus* were transterred to un-infected wood, the fungus did not develop in the latter. Since, in addition, colonization of stumps by *F. annosus* often precedes invasion by insects, it seems unlikely that this method of infection is important.

Although evidence given in an earlier paper (Rishbeth, 1950) suggests that *F. annosus* does not grow freely in soil or litter, it is conceivable that spores washed down through the soil might germinate on stump roots and infect them. The presence of viable *F. annosus* spores in soil at a depth of 50–90 cm. has been demonstrated (p. 10). It is difficult, however, to obtain mycelial growth of *F. annosus* on unsterilized root lengths in the laboratory after inoculation by spores. In addition, there is no field evidence that *F. annosus* becomes established on roots by means other than contact with roots already infected. This means of stump infection, therefore, is also probably unimportant.

If trees already in early stages of this root disease are cut down, *F. annosus* may enter the stumps from infected roots. This mode of infection will be discussed more fully in a subsequent paper.

SAPROPHYTIC ACTIVITY IN STUMPS

i. *Rate of growth*

A knowledge of the growth rate of *F. annosus* should indicate the length of time required for the fungus to occupy lateral roots from the exposed stump surface. Growth rates on malt agar of ten strains were determined over a range of temperatures and averages of the values obtained are recorded in Table X. The figures given are average increases in radius (mm.) per day of colonies.

TABLE X

Growth Rates of F. annosus *on Malt Agar: Average Values derived from Ten Strains*

Temperature (° C.)	0	5	10	15	22·5	25	28·5
Growth rate .	0·4	1·4	2·5	4·6	7·8	6·6	3·1

Individual strains do not differ greatly from one another over the range 5°–15° C., which is one most commonly found in Breckland soils. The values obtained are similar to those given by Cartwright and Findlay (1934), but do not necessarily correspond to growth rates of the fungus in pine roots. This was estimated by a method closely resembling that described in an earlier paper (Rishbeth, 1950) for studying interaction of fungi in roots. Pine roots of 0·6–0·8 cm. diameter, selected from healthy trees, were cut into 6 cm. lengths, washed, and almost stripped of bark scales. Twelve such unsterilized root lengths were attached to a pine-block inoculum containing *F. annosus*. After a suitable period of incubation, the root lengths were split longitudinally and re-incubated; the extent to which *F. annosus* had grown in them could then be measured. Allowance had to be made for an initial lag in growth rate as the fungus first entered the root lengths. By this method the highest growth rate recorded at 10° C. was 2·8 mm. per day and that at 22·5° C. 7·3 mm. per day. These agree well with the corresponding growth rates on malt agar given in Table X.

Observations were also made on the growth rate in stumps. A stump was infected by drilling four holes in the exposed surface and plugging them with a sawdust culture of *F. annosus*. After 7 months the mycelium had just started to grow along the lateral roots from the main body of the stump, and its position on five of them was recorded. Twenty-three weeks later the position of the mycelium on the same lateral roots was again recorded and the fungus was found to have grown an average distance of 43 cm. This gave a growth rate of 2·6 mm. per day or 1·0 m. per year. Since the mean soil temperature over the period was about 10° C., the value obtained agrees well with the growth rate in root lengths. The extent of growth along roots of naturally infected stumps about 2 years after felling was also determined. Assuming that the cut surface had been infected within a few weeks of felling, *F. annosus* was estimated to have grown at a maximum rate of 1·1 m. per year.

Since the standard planting distance for pines is about 1·4 m., it is clear that *F. annosus* growing on stump roots can closely approach adjacent trees within a year. Complete occupation of lateral stump roots, which may be from 2 to 4 m. long at the time of first thinning, must take several years. *F. annosus* can also colonize sinker roots to a depth of at least 2 m.

ii. *Infective capacity of stumps*

The first infections of living roots in contact with dead stump roots containing *F. annosus* were seen 1½–2 years after felling. Stumps are a potential source of infection as long as *F. annosus* remains in them; in large stumps the fungus is often found 15 years after felling and is occasionally still present after 30 years.

Field observations suggest that, around stumps containing the parasite, fewer living roots are infected in acid than in alkaline soils. As stated in an earlier paper (Rishbeth, 1950), growth of *F. annosus* on the surface of roots in acid soils is restricted, probably owing to the abundance of competing fungi. The lower incidence of infections on such roots may partly be explained in this way. It is also possible, however, that infections are fewer because of the relative infrequency with which *F. annosus* emerges from such stump roots. To discover whether this is so, roots containing *F. annosus* were taken from stumps in contrasting soils. Roots were washed, wrapped in moist paper, and incubated for a fortnight at 10° C. This low temperature was chosen to avoid undue interference from other fungi at the root surface. Under these conditions, *F. annosus* often broke through the surface to produce small, compact aggregations of mycelium. For each root, mycelial aggregations were counted and the approximate surface area calculated. Data obtained from a number of sites, from each of which several stump roots were taken, are given in Table XI.

TABLE XI

The Ability of Stump Roots containing F. annosus, *obtained from Various Sites, to produce Mycelial Aggregations on the Surface during Incubation at 10° C.*

Tree.	Period since felling (years).	Soil reaction.	Approx. total surface area of root (sq. dcm.).	No. of mycelial aggregations.	No. of aggregations per 10 sq. dcm.
Pine	4	Alkaline	46·3	84	18
,,	,,	Acid	29·7	27	9
,,	20–30	Alkaline	52·6	64	12
,,	,,	Acid	27·7	0	0
Birch	,,	Alkaline	28·1	26	9
,,	,,	Acid	21·6	6	3

The ability of roots to produce mycelial aggregations under these conditions probably gives a roughly comparative estimate of their infective capacity. If this is so, the infective capacity of stump roots in acid soils is in marked contrast to that of stump roots in alkaline soils, particularly with old pine stumps. This difference arises, most probably, from a greater abundance of competing fungi at the surface of stump roots in acid soils. The figures also suggest that infective capacity of stump roots falls off as the period after felling increases, decline being particularly evident with roots of pine stumps in acid soil. This decline, which implies a loss of vigour, is in all probability due to a gradual depletion of food by *F. annosus*.

iii. *Competition during colonization*

Many fungi are present in pine stumps soon after felling. Interaction between *F. annosus* and some of these other fungi was investigated by the method described in an earlier paper (Rishbeth, 1950). The results obtained suggest that *F. annosus* and other basidiomycetes can compete successfully with most moulds in recently cut stumps. *Trichoderma viride* Pers. ex Fr. rarely replaces *F. annosus* in large, freshly cut stumps, probably because the wood tends to be resinous. *F. annosus* replaces other basidiomycetes in stumps little if at all; the ability of *Peniophora gigantea* to replace *F. annosus* has already been mentioned.

The distribution of other fungi in freshly cut stumps suggests that they, like *F. annosus*, colonize stumps by air-borne spores. The thick bark of larger stumps appears to limit very considerably direct invasion of fungi from soil or litter. Tunnelling by insects eventually reduces the efficiency of this barrier, but fungi established by air-borne spores probably have an initial advantage. Some fungi present on the surface of living roots probably penetrate them after felling and thus compete with *F. annosus* when it grows down from the stump surface. *F. annosus* grows along stump roots already occupied by certain fungi, such as *Cylindrocarpon radicicola* Wollenw. and some causing 'blue-stain', but not along roots containing others.

iv. *Replacement of* F. annosus

In a preliminary experiment the ability of soil fungi to replace *F. annosus* was determined by burying lengths of naturally infected pine root. These were dug up at intervals, washed, split longitudinally, and incubated at 22·5° C. They were examined after a week and, if conidia of *F. annosus* were found, the fungus was known to be present; if they were not found, *F. annosus* was presumed to be absent. Results obtained in this way from three contrasting soil types are given in Table XII.

TABLE XII

The Approximate Survival Period of F. annosus *in Roots buried in Breckland Soils*

Former status of site.	Survival (weeks) at a depth of: 8 cm.	20 cm.
Arable (alkaline)	11	50
Woodland (alkaline)	25	110
Woodland (acid)	6	9

Here, other fungi replaced *F. annosus* more rapidly in the acid woodland soil than in either of the alkaline soils. In a similar experiment *F. annosus* tended to survive longer in naturally infected roots if they were resinous.

The ability of ten East Anglian strains of *Trichoderma viride*, mentioned in an earlier paper (Rishbeth, 1950), to replace *F. annosus* in root lengths under controlled conditions was tested. Three types of root were used: some were selected from stumps well rotted by *F. annosus*, others were taken from trees being invaded by the fungus and were very resinous, and the remainder were roots freshly infected with *F. annosus* in the laboratory. All were of similar thickness and were cut into 5-cm. lengths. To prepare inocula of *T. viride*, root sections of about 3 mm. thickness were sterilized and placed on malt agar cultures of the various strains; they were ready for use after incubation at 22·5° C. for a week. One such inoculum was pinned on to the end of each root length, which was then placed in a moist, sterile test-tube.

Root lengths of the types described, inoculated singly with the ten strains of *T. viride* and placed in tubes, were stored in covered containers at 5°, 10°, and 15° C. After a suitable period lengths were tested for the presence of *F. annosus* in the manner already described. The number of strains of *T. viride* which replaced *F. annosus* under these conditions is given in Table XIII.

TABLE XIII

The Interaction of Ten Strains of T. viride *with* F. annosus *in Lengths of Pine Root of Various Types*

Storage		No. of strains replacing *F. annosus* in the following types of root length		
Temp. (° C.)	Duration (weeks).	Naturally infected, rotted.	Naturally infected, resinous.	Artificially infected.
15	2	10	2	4
10	4	6	0	2
5	6	2	0	1

Root lengths infected with *F. annosus* in the laboratory were intermediate between resinous and rotted ones in their ability to prevent invasion by *T. viride*. The result obtained with rotted root lengths at 15° C. suggests that *T. viride* can replace *F. annosus* rapidly from old rotted stumps during the summer. On the other hand, resinous roots containing *F. annosus* are unlikely to be invaded by *T. viride* in winter. The infrequency of *T. viride* in large, freshly cut pine stumps may partly be attributed to the resinous wood.

It has been shown in an earlier paper (Rishbeth, 1950) that, under experimental conditions, *F. annosus* is readily replaced in rotted wood by many other fungi. The succession of fungi in rotted pine stumps was not worked out in detail but certain observations were made. *F. annosus* is often replaced by *Trichoderma viride* and also to some extent by *Torula ligniperda* (Willkom) Sacc. Such fungi are closely followed by others, amongst which *Hypholoma fasciculare*, *Melanospora* sp., and various 'blue-stain' fungi were noted: these were not found replacing *F. annosus* directly. The relation of zone-lines to succession will be considered later.

To determine whether *T. annosus* is replaced more rapidly in some sites than in others, stumps of known history containing the fungus were examined in thinned and clear-felled plantations. The main lateral roots were exposed as far as the characteristic rot extended and were brought back to the laboratory. For each root the total length containing *F. annosus* was determined by incubating it in sections. Great variation was found in stumps of the same age: some had the fungus present in all main lateral roots, whereas others had it only in sinker roots. Table XIV records the amount of *F. annosus* present in the lateral roots of these stumps.

In these stumps the appearance of the wood strongly suggested that most roots without *F. annosus* once contained it. *F. annosus* was replaced more extensively in acid soils than in alkaline ones, both in stumps left by thinning (sites A and B) and in those left by clear felling (sites C and D). The slow rate of replacement of *F. annosus* in alkaline soils is clearly shown by its prevalence in stumps of site C, 25 years after felling. These differing extents of replacement agree with the results given in Table XII and are almost certainly related to differences in soil microflora, referred to in an earlier paper (Rishbeth, 1950). The fact that *F. annosus* tends to survive longer in sinker roots than in laterals is probably due to reduced competition from other fungi at lower levels in the soil.

TABLE XIV

The Amount of F. annosus *present in Lateral Roots of Stumps in Contrasting Soil Types*

Former status of sites: A Arable (alk.); C Woodland (alk.); B Heath (acid); D Woodland (acid).

Site	A	B	C	D
Age at felling (years)	10	15	*c.*60	*c.*60
Period since felling (years)	10	10	25	25
Total No. of stump roots examined	13	30	14	15
No. of roots with *F. annosus*	11	12	12	6
Total length of roots with *F. annosus* (m.)	20·2	7·85	14·7	4·2

v. *Zone-lines*

Roots in which replacement has occurred are often marked by a series of zone-lines, the latest one formed separating *F. annosus* from one or more invading fungi (Pl. I, E). Irregular zone-lines are also commonly present in wood below the surface of stumps. The presence of such lines in wood has been noted by many workers: Brooks (1915), for instance, described conspicuous black zones caused by *Ustulina zonata* in the wood of the rubber tree. Hopp (1938) stated that zone-lines are produced in very specific situations where hyphae in wood are exposed simultaneously to large amounts of water and air.

The conditions under which zone-lines are produced in pine roots were investigated. When lengths of freshly infected or rotted root containing *F. annosus* were stored in a saturated atmosphere at various temperatures, no zone-lines were formed. Other root lengths of various sizes were stored at 70 per cent. relative humidity and at about 20° C. After 2 months several larger root lengths (diameter 2·0 cm.) were found to contain zone-lines at each end, whereas smaller ones (diameter 0·5 cm.) had none. When similar small root lengths were embedded in paraffin wax, leaving only the upper part exposed to partial desiccation at 70 per cent. R.H., zone-lines were often formed about 0·5 cm. below the exposed surface (Pl. I, F). The failure of small root lengths not embedded in wax to form zone-lines was thus probably due to rapid drying out. These results agree with the observations of Hopp (1938) on various wood-rotting fungi in wood blocks.

Stump surfaces tend to dry out to some extent soon after felling, and so, with stumps containing *F. annosus*, the first zone-line may be formed beneath the surface at a relatively early stage. Since intact bark provides considerable protection from desiccation, larger roots probably dry out to an extent sufficient to initiate formation of zone-lines only when they have been partially rotted by *F. annosus*. A high oxygen tension is required for formation of zone-lines (Hopp, 1938), so for this reason also they are more likely to be produced in partially rotted roots, allowing relatively free gaseous diffusion. The sandy East Anglian soils fluctuate considerably in water content: in winter they often contain over 10 per cent. moisture whereas in summer they may have as little as 2 per cent. Rotting stump roots thus probably dry out in stages, which would account for the formation in them of zone-lines at irregular intervals.

The ability of *Trichoderma viride* and *Torula ligniperda*, which replace *F. annosus* in roots, to penetrate *F. annosus* zone-lines was also investigated. Experiments were performed with large root lengths containing *F. annosus* which had been buried for a period and had marked zone-lines at each end. In some of these the zone-lines were deliberately damaged. A wood inoculum containing one or other of the above-mentioned fungi was placed on one end of each root length. Root lengths with inocula in position were incubated in moist containers at about 20° C. for 10 days. Other root lengths containing *F. annosus* of similar size but having no zone-lines were treated in the same way. *F. annosus* was not replaced in root lengths with intact zone-lines, whereas extensive replacement, both by *Trichoderma viride* and *Torula ligniperda*, occured in roots in which zone-lines were absent or had been damaged. This clearly suggests that zone-lines of *F. annosus* constitute at least a temporary barrier to growth of other fungi along stump roots. A similar conclusion was drawn by Campbell (1933) with respect to invasion by bacteria and fungi of zone-lines produced by *Xylaria polymorpha* in a beech trunk.

The probable relation of zone-lines to succession in stumps containing *F. annosus* is now clearer. A zone-line formed in a stump root as a result of partial desiccation cuts off *F. annosus* in the portion distal to it from the extensive food-base comprising most of the stump. Even if *F. annosus* remains viable there, it may be replaced by other fungi when the tissues become moist again. In alkaline soils, where fungi capable of entering such roots are comparatively scarce, *F. annosus* often survives for a long time. In acid soils fungi such as *Trichoderma viride* enter the root portion distal to the zone-line where they may in turn be replaced by others, such as *Hypholoma fasciculare* and certain 'blue-stain' fungi. These saprophytes cannot pass a recently formed zone-line, however, and are temporary halted. When the root dries out again, they penetrate the zone-line, possibly because it is damaged by expansion and contraction of the wood. Meanwhile, drying out has caused *F. annosus* to form another zone-line nearer the body of the stump; this in turn temporarily checks the advance of other fungi. A similar process occurs in the wood below the stump surface, so that, in large roots, *F. annosus* may eventually be replaced both from the body of the stump and from the distal portion of the root. An interesting situation thus arises where succession occurs in stages as a result of the characteristic reaction of *F. annosus* to drying out. If it were not for this reaction, replacement of *F. annosus* by other fungi in stumps would almost certainly be more rapid.

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LITERATURE CITED

BAVENDAMM, W., and REICHELT, H., 1938: Die Abhängigkeit des Wachstums holzzersetzender Pilze vom Wassergehalt des Nährsubstrates. Arch. Mikrobiol., ix. 486.

BJØRNKÆR, K., 1938: Undersøgelser over nogle danske Poresvampes Biologi med saerligt Hensyn til deres Sporefældning. Friesia, ii. 1.

BROOKS, F. T., 1915: A Disease of Plantation Rubber caused by *Ustulina zonata*. New Phyt., xiv. 152.

—— and MOORE, W. C., 1923: On the Invasion of Woody Tissues by Wound Parasites. Proc. Camb. Phil. Soc. (Biological Sciences), i. 56.

—— —— 1926: Silver-leaf Disease. V. J. Pomol. and Hort. Sci., v. 61.

CAMPBELL, A. H., 1933: Zone Lines in Plant Tissues. I. The Black Line formed by *Xylaria polymorpha* (Pers.) Grev. in Hardwoods. Ann. Appl. Biol., xx. 123.

CARTWRIGHT, K. ST. G., and FINDLAY, W. P. K., 1934: Studies in the Physiology of Wood-destroying Fungi. II. Temperature and Rate of Growth. Ann. Bot., xlviii. 481.

GARRETT, S. D., 1944: Root disease fungi. Waltham, U.S.A.

GREGORY, P. H., 1945: The Dispersion of Air-borne Spores. Trans. Brit. Mycol. Soc., xxviii. 26.

HOPP, H., 1938: The Formation of Colored Zones by Wood-destroying Fungi in Culture. Phytopath., xxviii. 601.

LEACH, R., 1939: Biological Control and Ecology of *Armillaria mellea* (Vahl) Fr. Trans. Brit. Mycol. Soc., xxiii. 320.

NAPPER, R. P. N., 1934: Root Disease Investigations. Rep. Rubb. Res. Inst. Malaya, 1933. 105.

PETCH, T., 1921: The Diseases and Pests of the Rubber Tree. London.

RISHBETH, J., 1950: Observations on the Biology of *Fomes annosus*, with Particular Reference to East Anglian Pine Plantations. I. The Outbreaks of Disease and Ecological Status of the Fungus. Ann. Bot., N.S., xiv. 365.

EXPLANATION OF PLATE I

Illustrating J. Rishbeth's paper on 'Observations on the Biology of *Fomes annosus*, with Particular Reference to East Anglian Pine Plantations. II. Spore Production, Stump Infection, and Saprophytic Activity in Stumps'.

A. Sporophore of *F. annosus* on a pine stump left by thinning: the white margin is unusually prominent. (× ⅕.)

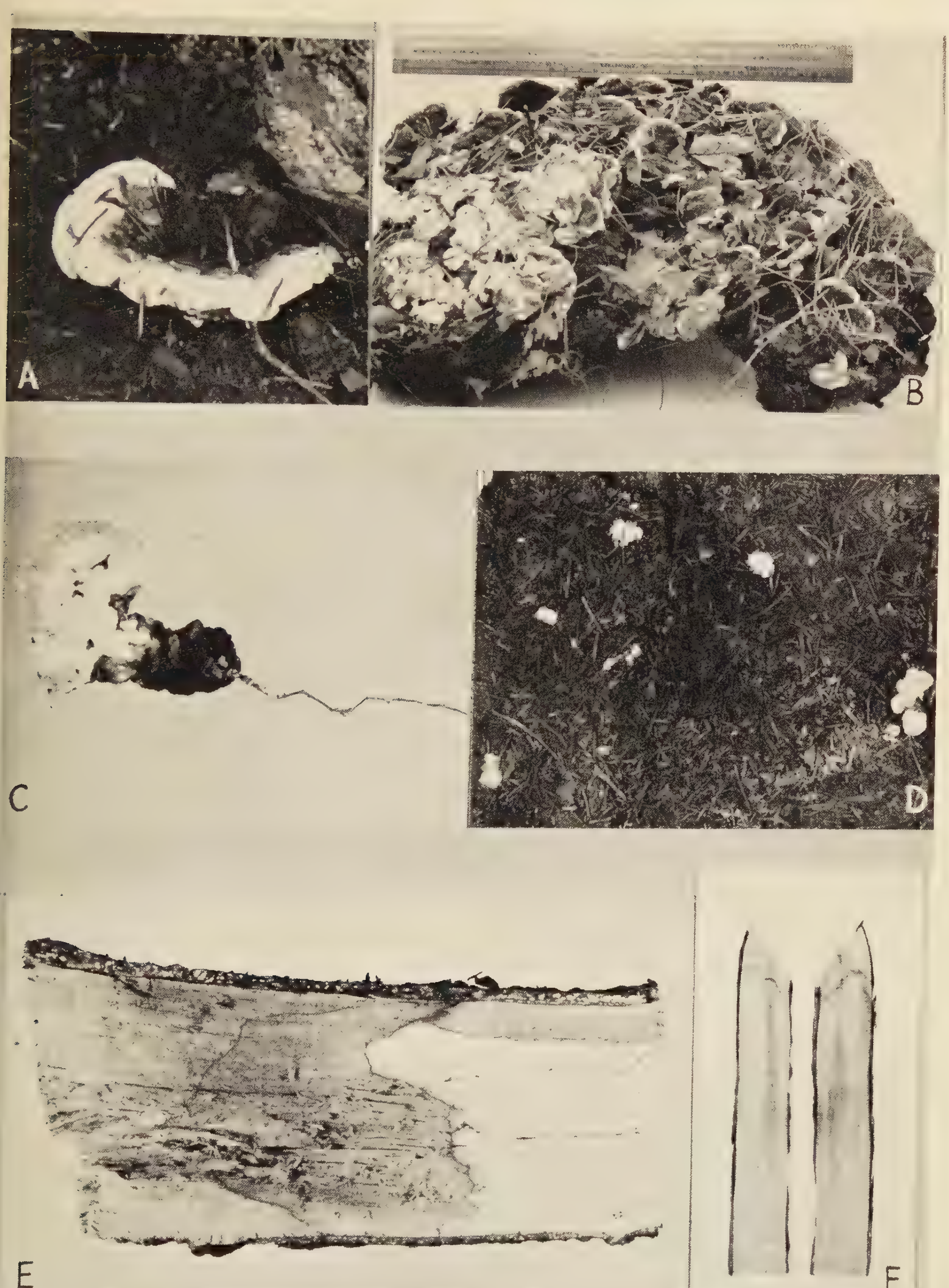
B. Massive imbricate sporophore of *F. annosus* from the base of a hawthorn bush. (× ⅕.)

C. Sporophore of *F. annosus* from litter near a dead pine, showing attachment to a small rotted root. (× ½.)

D. Similar small sporophores lying in litter. (× ⅖.)

E. Zone-lines in a birch stump root. *F. annosus* was still present in the lightest area (right), but had been replaced by other fungi in the remainder of the root. (× ½.)

F. A length of pine root split longitudinally to show zone-line formation following storage at 70% relative humidity for 2 months. (× 1.)



J. RISHBETH

Growth and Heterocyst Production in *Anabaena cylindrica* Lemm.

III. The Cytology of Heterocysts.

BY

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With Plates II–IV and one Figure in the Text

SUMMARY

A study has been made of the cytology of the heterocysts of *Anabaena cylindrica* Lemm. at different stages during the growth of this alga in culture. The techniques used include that of Feulgen, a modified Sakaguchi test for arginine derivatives, ultra-violet photomicrography, and micro-incineration. It has been shown that the formation of a heterocyst begins with the mobilization of material so that its protoplasm becomes homogeneous. The protoplast subsequently becomes depleted of all materials demonstrable by the methods used. At intermediate stages in development substances become concentrated near the connexions of the heterocyst and a central transverse zone, retaining material longer than the rest of the protoplasm, becomes apparent. The structure and development of heterocysts is discussed.

OUR knowledge of the cytology of the heterocysts of the Myxophyceae, reviewed by Fritsch (1945), is largely based upon observations made with material of ill-defined physiological condition and there appear to be several techniques which might be of value which have not hitherto been used in the study of these structures. In view of this, it has seemed desirable to supplement researches on the physiology of heterocyst formation (Fogg, 1944, 1949) with the cytological investigations described in this paper.

MATERIAL AND METHODS

Anabaena cylindrica Lemm. was grown as in previous investigations of this series in pure culture in a mineral salt solution containing no combined nitrogen (Fogg, 1949). Most of the material used was derived from a single culture series which was sampled at increasing intervals of time after inoculation. These cultures were made in 500-ml. portions of medium contained as 2-cm. layers in 'Hysil' vessels, and were inoculated from an 11-day-old stock culture. They were arranged at equal distances around a 100-watt pearl lamp in a light intensity, measured in the plane of the surface of the medium, of *c.* 1,500 metre-candles. The temperature, which was not controlled, had a mean value of 28° C. A single flask was taken for each sample, variation

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between replicate cultures having been found negligible. Measurements of the characteristics given in Table I were made (for methods see Fogg, 1949) and show that events in these cultures followed a course described in more detail elsewhere (Fogg, 1944). The alga was then centrifuged down and fixed with formaldehyde at a final concentration of 4 per cent. With the exception of those made by ultra-violet photomicrography, observations on this set of samples have been repeated and confirmed with material from other culture series.

TABLE I

Day.	Density of alga; μ filament per mm.³ of medium.	Mean relative growth constant; R.	Mean cell length; μ.	% heterocysts.
0*	137	—	4·23	2·70
1	217	0·460	4·04	0·71
2	486	0·806	3·36	4·45
4	3,390	0·971	3·53	3·12
8	13,060	0·338	3·51	4·02
23	31,800	0·059	3·33	3·02
38*	53,750	0·035	4·28	3·99

* Akinete formation just beginning.

Except where otherwise stated, drops of algal suspension were dried on slides *in vacuo* at room temperature for examination, Haupt's adhesive (Johansen, 1940) or celloidin (Baker, 1947) being used to attach the filaments to the glass. The material was examined by the following principal methods:

Haematoxylin staining. For general comparative purposes preparations were stained with Heidenhain's haematoxylin (Johansen, 1940).

Feulgen staining. Poljansky and Petruschewsky (1929) have shown that this method applied to blue-green algae indicates the presence of material containing organically bound phosphorus and taking up nuclear stains and therefore containing desoxypentose nucleic acid and similar to chromatin. The procedure used in this work was that described by Johansen (1940). It was found that hydrolysis was best carried out for 5 minutes in N hydrochloric acid at 60° C.

The Sakaguchi test. The most successful of the methods examined for the cytochemical demonstration in *Anabaena* of nitrogenous material, the distribution of which is of particular interest in relation to the results described in previous papers of this series, is that of Sakaguchi as modified by Baker (1947). Baker has shown that the method can be taken as specific for arginine, an amino acid which was found to be present in substantial amounts in the blue-green algae examined by Mazur and Clarke (1938, 1942). Since the free substance must diffuse out from fixed material, a positive result most probably indicates the presence of protein, of which arginine appears to be a universal constituent.

Ultra-violet photomicrography. The high resolution obtainable by this method has been particularly valuable for the elucidation of the minute structural details of *Anabaena* heterocysts. The apparatus employed was the new Cooke, Troughton, and Simms ultra-violet microscope recently installed in the Botany Department of the University of Leeds. The only essential difference between this instrument and the prototype described by Barnard and Welch (1936) lies in the mechanism of the fine adjustment. The Zeiss quartz lenses used are computed to work with the 2,750 A line of the cadmium arc. Since fluorescence of the algal pigments in ultra-violet light was expected, the material was first decolorized by extraction overnight with 95 per cent. alcohol at room temperature. It was mounted for examination in 25 per cent. aqueous glycerol. Photographs were taken at an initial magnification of 1,000 diameters, in focal planes at intervals of 0·5 μ through the specimen, and subsequently enlarged to a final magnification of 3,000 diameters; this represents the limit of significant magnification with the wave-length used. Since photographs were taken at only the one wave-length, the relative absorption shown by any particular region of the cell cannot be taken as a reliable indication of its chemical composition.

Micro-incineration. The inorganic content of heterocysts was investigated by micro-incineration. Living material was suspended in distilled water and drops of the suspension dried on to cover-glasses. After recording the positions of a number of heterocysts, incineration was carried out on silica plates at *c.* 500° C. in the manner described by Horning (in Bourne, 1942).

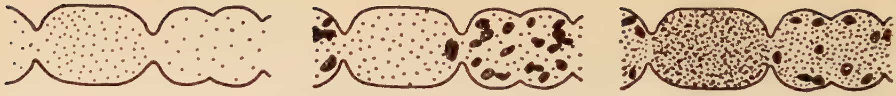
RESULTS

(*a*) *Normal cells*

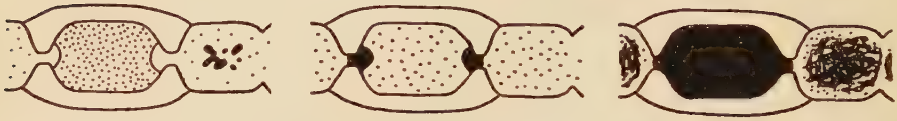
This investigation has yielded little new information concerning the normal cells of Myxophyceae, but several features in their structure must be commented on before the observations on heterocysts are described.

Centroplasm. In the ultra-violet photomicrographs of mature cells from slowly growing cultures, i.e. samples 8, 23, and 38, the centroplasm is distinguishable by its higher absorption, although its boundary with the chromatoplasm is always more or less diffuse (Pl. II, Figs. 1, and 6, Pl. III, Figs. 7, 9, and 10). The centroplasm usually appears to be irregularly granular, but in some of the photographs there is a suggestion of an alveolar structure (Pl. I, Fig. 6). Feulgen preparations from the same samples showed a variable number of densely staining granules (Text-fig. 1), apparently without interconnecting strands, in the centroplasm. In haemotoxylin preparations the whole centroplasm was stained, granules within it taking up the colour more intensely. The Sakaguchi reagent imparted a faint pink colour to the protoplast in general but did not differentiate its two main parts.

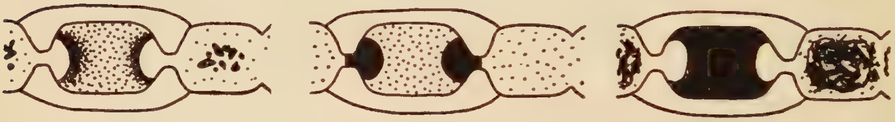
The absorbing material within the centroplasm shown in the ultra-violet photomicrographs appears to be that staining with the Feulgen reagent and with haematoxylin, and thus possesses some at least of the properties of chromatin. It is possible that it forms a spongy structure the finer portions of which are below the limits of resolution of the visual light microscope and which are only evident in favourable cases in a few of the ultra-violet photomicrographs. If these suppositions are correct, then the observations



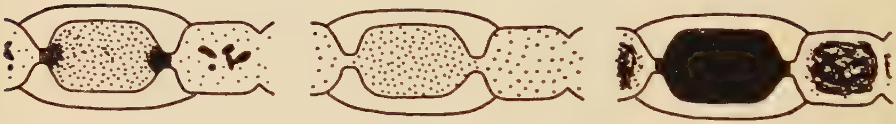
(a) 1st Day



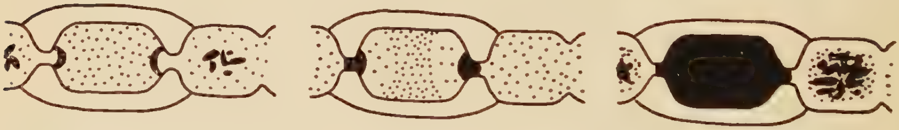
(b) 2nd Day



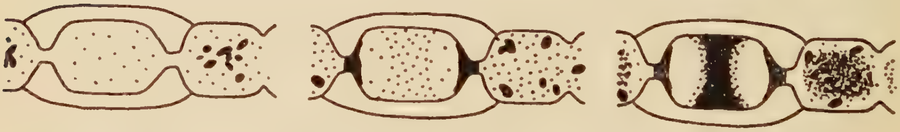
(c) 4th Day



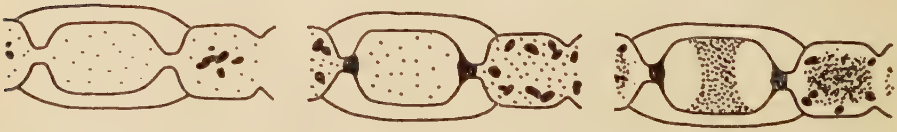
(d) 4th Day (alternative forms)



(e) 8th Day



(f) 23rd Day



(g) 38th Day

TEXT-FIG. 1. Diagram showing typical Feulgen, Sakaguchi, and Heidenhain haematoxylin staining reactions of heterocysts of *Anabaena cylindrica* at different stages during growth in culture.

described are in line with those of Poljansky and Petruschewsky (1929) and of Spearing (1937), who found that the centroplasm of blue-green algae contains a substance similar to chromatin in the form of a reticulum or sponge.

Of the samples taken during the early stages of growth, that obtained at one day contained material which was beginning rapid growth after a lag phase of about 12 hours. In an ultra-violet photomicrograph of this material the centroplasm and chromatoplasm are no longer distinct (Pl. II, Fig. 3). Similarly in haematoxylin preparations the protoplasts stained uniformly without differentiation of centroplasm. It has been shown (Fogg, 1944) that at the beginning of exponential growth the cells of *Anabaena* contain a markedly higher concentration of combined nitrogen than at other times. Caspersson (1947) has described how, in the initial stages of active growth, the cells of various organisms show high concentrations of cytoplasmic nucleotides, substances which absorb strongly ultra-violet light of wave-length 2,750 A, these concentrations paralleling the rate of protein synthesis. It thus seems likely that the heavy absorption towards ultra-violet light and the general staining with haematoxylin shown by the protoplast at this stage in the growth of *Anabaena* is to be ascribed to nucleotides. Granules staining with the Feulgen reagent were rarely observed in the centroplasm at this stage, although exactly the same staining procedure was used as for the older material. The evidence from haematoxylin and Feulgen staining thus points to a dispersion or diminution of the chromatin of the centroplasm during the lag. In samples 2 and 4, taken during the exponential phase of growth, the centroplasm presented an appearance more resembling that in slowly growing material (Pl. II, Fig. 4).

Cyanophycin. Granules colouring intensely with the Sakaguchi reagent and with haematoxylin were conspicuous in the chromatoplasm of the material used for inoculum and that from the later stages of culture, i.e. in samples 0, 1, 23, and 38, but were absent during rapid growth (Text-fig. 1). These could not be demonstrated after extraction of the alga for 2 hours with N hydrochloric acid at room temperature and thus appear to have been cyanophycin (Fritsch, 1945, p. 787). The nature of the other kinds of granular inclusions occurring in the normal cells was not investigated.

Cell membranes. In ultra-violet photomicrographs taken at all stages of growth the inner investment of the trichome shows as a highly absorbent membrane, of the order of 0·1 μ in thickness, uninterrupted along the length of the filament (see especially Pl. II, Figs. 3 and 6). A mucilaginous sheath is not visible. The septa did not absorb ultra-violet appreciably and appear as unperforated plates, about 0·2 μ in thickness. This very different absorption shown by the septum makes it certain that it differs fundamentally in composition from the longitudinal membrane, a fact that has been deduced from its microchemical properties (Ullrich, 1930; Mühldorf, 1938). Mühldorf's assertion that plasmodesmata are absent from the Myxophyceae receives confirmation in this case.

(*b*) *Akinetes*

An ultra-violet photomicrograph of two akinetes is reproduced in Pl. II, Fig. 11, but these structures were not investigated further.

(*c*) *Heterocysts*

Considerable variation in the structure and contents of heterocysts during the development of cultures has been observed. This is evident from the photographs and from Text-fig. 1, which presents in diagrammatic form for each of the samples the most typical results obtained with the three stains used.

In preparations stained with haematoxylin seven principal forms of heterocyst could be distinguished, as follows:

A. Cells in the first stage of transformation into heterocysts, as shown by their elongated shape and constricted connexions, stained rather more deeply than did normal cells. In most cases the protoplast appeared homogeneous, granules being rare, but in about one-third of the cases examined the centroplasm was differentiated by slightly deeper, but uniform, staining. The sheath was absent or of not more than half its final thickness (Text-fig. 1, *a*). Cells such as this have not been counted as heterocysts in previous work (Fogg, 1944, 1949) or in arriving at the frequencies given in Table I.

B. Distinguished by intense and uniform staining of the protoplast. Sheath fully developed and connexions narrow (Text-fig. 1, *b*; Pl. III, Fig. 8).

C. Similar to B except for a prominent unstained region at either pole (Text-fig. 1, *c*).

D. Deeply stained at the poles and in a central transverse zone, with intervening unstained regions (Text-fig. 1, *f*; Pl. II, Fig. 2).

E. Distinguished from D by the absence of the deep staining at the poles.

F. Distinguished from D by the absence of the deeply staining transverse zone. Stages intermediate between D and F were frequent (Text-fig. 1, *g*).

G. Showing no internal structure in haematoxylin preparations.

The proportions to these types occurring at different stages during growth of the cultures are given in Table II. A total of about 50 heterocysts was counted in each sample. The sequence in which the different types predominated supports the idea that they represent a developmental series from type A, the earliest stage, through to type G, the senescent form. From the data available it is possible to form a rough idea of the rate at which development occurred. It is clear that type A was transient, lasting for only a few hours. Total numbers of heterocysts of types B and C and of types D, E, F, and G, calculated from the data of Tables I and II, plotted against time gave curves suggesting that the duration of stages B and C together was of the same order as the generation time of normal cells, increasing from about 12 hours in young material to 10 or more days in old cultures.

TABLE II

Sample.	Percentages of total number of heterocysts.						
	A.	B.	C.	D.	E.	F.	G.
0	0	10	6	37	31	6	10
1	47	20	5	5	5	12	5
2	7	85	0	7	0	0	2
4	0	32	59	0	8	0	0
8	0	70	15	4	9	2	0
23	0	0	3	40	9	18	27
38	0	7	7	30	16	14	26

The successive samples showed a regular change in the content of Feulgen-staining material of heterocysts as compared with that of normal cells. The protoplasts of young heterocysts of type A from sample 1 stained uniformly, slightly more deeply than the pale colour imparted to normal cells in the same material. This differentiation became more marked in samples 2 and 4. In material from sample 8 the general staining of the majority of heterocysts was reduced and in subsequent samples these structures were mostly unstained (Text-fig. 1). Deeply staining granules such as those in the centroplasm of a normal cell were never found at any stage in development. Most heterocysts in sample 2 had an unstained region at either end (Text-fig. 1, *b*). This was still present in many cases in sample 4, but was rendered more prominent by a deeply staining zone bordering it internally (Text-fig. 1, *c*). Others in this sample had no unstained region, the deeply staining material extending up to the connexion (Text-fig. 1, *d*). In sample 8 the extent of polar staining was reduced and it was absent in the later samples (Text-fig. 1, *f* and *g*). The central transverse zone present in haematoxylin types D and E was never observed to be stained in Feulgen preparations.

The Sakaguchi reagent did not stain type A heterocysts any more deeply than it did normal cells. However, as the cultures developed, the general staining with this reagent of these structures relative to that of normal cells increased somewhat in intensity up to the fourth day and subsequently declined. The extent of deeply stained regions at either pole underwent changes parallel to this. In sample 4 these regions were extremely prominent in most cases, although some heterocysts were entirely without them (Text-fig. 1, *c* and *d*). These two kinds seem to correspond with types C and B distinguished in the haematoxylin preparations. This differentiation at the poles persisted in most heterocysts of sample 38 but was much reduced in extent. A central transverse zone corresponding to that in the haematoxylin types D and E was more deeply stained than adjacent regions in samples 8 and 23 (Text-fig. 1, *e* and *f*). In the heterocysts from sample 38 the bulk of the protoplast was unstained (Text-fig. 1, *g*). After extraction with N hydrochloric acid for 2 hours at room temperature, heterocysts retained their staining reactions with the Sakaguchi reagent.

A series of ultra-violet photomicrographs of heterocysts is reproduced in the plates. That from sample 1 (Pl. II, Fig. 3) shows the characteristic form of a heterocyst of type A. The protoplast is uniform and nearly free from granules and has a high absorption nearly equal to that of normal cells from the same sample. Pl. II, Figs. 4, and 5, Pl. III, Fig. 9, from samples 2, 4, and 8 respectively, show that the protoplasm of a heterocyst retained its high absorption until after the full development of the sheath. The bulk of the protoplast at this stage is seen to be more or less homogeneous, free from granular inclusions, and showing no aggregation of absorbent material as in the centroplasm of normal cells. This corresponds with the uniform and intense staining with haematoxylin. Two of the samples, depicted in Pl. II, Fig. 4, Pl. III. Fig. 9, show less absorption in the peripheral regions of the protoplast. It is not clear whether this represents a remnant of the division into centroplasm and chromatoplasm or the beginning of a general decrease in absorbing material within the heterocyst. The contents of heterocysts from the older cultures showed little absorption in comparison with those of normal cells in the same material (Pl. III, Figs. 10 and 11).

There are indications in the ultra-violet photomicrographs of differentiation of zones at the poles corresponding to that demonstrated by staining (Pl. II, Fig. 5, Pl. III, Fig. 9). A central transverse zone is shown clearly in several photographs (Pl. II, Fig. 1, Pl. III, Figs. 7 and 10) by reason of its higher absorption compared with the rest of the protoplast. In two instances (Pl. II, Fig. 1, Pl. III, Fig. 10) it appears finely granular. This zone extends fully across the heterocyst up to the inner investment, its boundaries on either side with the rest of the protoplast being more or less diffuse. There appears to be a decrease of absorbing material within it as the heterocyst ages, since this feature was only faintly discernible in an old heterocyst from sample 38 (Pl. III, Fig. 11).

The ultra-violet photomicrographs show the structure of the connexions more clearly than is possible with the visual light microscope. Pl. II, Fig. 3, illustrates a stage in the deepening of the constriction between two cells which results in the formation of the narrow connexion seen in mature heterocysts. Pl. II, Fig. 3, in conjunction with other photographs taken at a series of focal levels through the same specimen, shows particularly clearly the absence of any protoplasmic connexions, of a size within the resolving power of the microscope, between the heterocysts and its neighbouring cells. The connexion finally consists of a tube formed by the inner investment (see Pl. III, Fig. 10). The lumen, which is about 0·2 μ in diameter, appears generally to contain no material of high absorption, perhaps no more than the remnants of the septum, but it is uncertain from the photographs whether or not at certain stages more absorbent matter may be present.

The inner investment of heterocysts is of the same thickness and absorbing power as that of normal cells. The sheath appears structureless in the ultra-violet photomicrographs, although the distinction between the inner 'cellulose' layer and the outer mucilaginous part was clear in other preparations.

Micro-incineration tests were not made with the samples on which most of the work that has so far been described was done, but on fresh material. Pl. III, Figs. 12 and 14, depict filaments from a 27-day-old culture, in which

51 per cent. of the heterocysts were found to be of type D in haematoxylin preparations, after drying and before incineration. It will be observed that the protoplasts of the heterocysts shrank to a greater extent on drying than did those of normal cells, their diameter in some cases being reduced to about one-third of that of the latter. In many instances (see Pl. IV, Fig. 14) the central part of the protoplast contracted less than the ends. This provides confirmation of the existence in some heterocysts of a central part with a greater content of dry matter than the end regions. The same filaments after incineration are shown in Pl. IV, Figs. 13 and 15. Whereas, with rare exceptions, each normal cell left a substantial inorganic residue, the heterocysts left no appreciable amount of ash. In repeated tests it has always been found that in cultures past the exponential phase of growth, 80 per cent. or more of the heterocysts leave no visible residue on ashing. On the other hand, in younger material, for example in a 2-day-old culture growing exponentially with 85 per cent. of the heterocysts in stage B in haematoxylin preparations, these structures were found to leave some ash on incineration, although not so much as the normal cells in the same material.

Tests made with sulphuric acid and iodine on *Anabaena* at different stages of development showed that the sheath gave a slight cellulose reaction from the earliest stages, the reaction becoming more definite as the heterocysts developed.

DISCUSSION

In *Anabaena cylindrica* heterocyst formation begins with the elongation of a cell somewhat beyond the limit at which it would otherwise divide. There is no appreciable increase in the diameter of the protoplast in this species. At the same time granular inclusions disappear and a sheath is formed. None of the methods used has revealed any marked differentiation within the protoplast of a heterocyst at this stage. The concentrations in the protoplasm generally of materials, probably proteins and nucleotides or their derivatives, demonstrable by nuclear stains, by the Sakaguchi test, or by their ultra-violet absorption, are then higher relative to those in normal cells. It is not clear whether this is solely due to the redistribution of substances previously concentrated in granules or whether synthesis also takes place.

There follows a migration of material from the heterocyst. Zones of high concentration of various substances form at either end of the cell, increase in extent, then decrease, while the bulk of the protoplast shows progressively weaker affinity for stains and decreasing ultra-violet absorption. It appears that arginine and Feulgen-staining material may at one stage be accumulated in separate regions, that containing the former substance being first established and the other arising at its inner margin. That this is so is suggested by a comparison of material from sample 4 stained by different methods. The region at either pole with no affinity for nuclear stains appears to correspond with that deeply coloured by the Sakaguchi reagent. It is to be expected that such a preponderance of the basic substance arginine would render this part unstainable with basic dyes. Material stainable with the Feulgen reagent accumulates at the inner margin of this zone. As the heterocyst develops these two zones apparently merge, then decrease in extent, and finally disappear. That materials other than those investigated in this study also migrate in this manner is suggested by the conspicuous amounts of potassium to be found at the poles of heterocysts at certain stages (Fogg, 1944). It seems reasonable to conclude that the substances accumulating in the region of the connexions eventually pass through into the neighbouring cells, but no cytochemical evidence of their arrival has been obtained. All the methods of examination used in this investigation agree in showing the protoplast of a mature heterocyst to be greatly depleted in comparison with that of a normal cell. A central transverse zone, however, retains certain materials, including those containing arginine, having affinity for haematoxylin, and with high ultra-violet absorption, for longer than other regions of the protoplast. This zone gives the impression of being one not reached by some mobilizing influence spreading from the connexions, but, although it too gradually loses material so that eventually it becomes indistinct, it does not decrease in extent as the heterocyst ages.

The initial stages of heterocyst formation in *Anabaena cylindrica* are similar to those that have already been described for other species of blue-green algae (e.g. by Geitler, 1921, and Bharadwaja, 1933). Various isolated observations by other workers are in accord with the subsequent developmental sequence outlined in this paper. Thus Poljansky and Petruschewsky (1929) found in many cases a diffuse and uniform staining with the Feulgen reagent of the contents of heterocysts of *Tolypothrix tenuis*, similar to that described for *Anabaena cylindrica* on p. 29. Bharadwaja (1933) recorded an intense staining with basic dyes of the contents of young heterocysts, like that illustrated in Pl. III, Fig. 8. It is generally accepted that fully developed heterocysts are depleted, at least in certain materials, and certain workers, notably Kohl (cited by Geitler, 1921), have given cytological evidence that these cells are degenerate. However, no thorough investigation of the later stages in development have hitherto been made and accounts of the structure of mature heterocysts are conflicting.

A major point of disagreement is that concerning the extent of differentiation within the protoplast. Geitler (1921) agreed with Baumgärtel (1920) that the structures present in normal cells, including those in the centroplasm, are also present in fully developed heterocysts. Poljansky and Petruschewsky (1929) and Spearing (1937), however, found no chromatic material like that in normal cells in these structures. This discrepancy can hardly be accounted for by differences between the forms investigated, since although those used by the different workers were not the same, a variety of species was studied in each case. Poljansky and Petruschewsky and Spearing, however, employed more critical methods than the earlier workers and their results have been fully confirmed in this investigation.

Polar granules are a prominent feature of heterocysts in many blue-green algae and have been usually supposed to consist of cyanophycin (Fritsch, 1945, p. 798). Refractive granules of this sort have been observed occasionally in *Anabaena cylindrica*, but since they were not soluble in dilute acid they cannot have been cyanophycin. Their nature has not been investigated further. The regions adjacent to the connexions in the heterocysts of this species have been found at certain stages to be differentiated by particularly high contents of certain substances. Since the boundaries of these regions are often diffuse and since they are characterized in different cases by different substances, it seems preferable not to call them granules. These features are clearly of the same nature as the plug-like *verschlusskörper* of Kohl which Geitler (1921) regarded as being of the same composition as the granules referred to above. Geitler's view does not appear to be a correct one, but it might be that under certain conditions, for example if the connexions with adjacent cells become severed or blocked, or with some methods of fixation, a substance concentrated near the connexion becomes aggregated to form a granule. Baumgärtel (1920) and Geitler (1921) considered polar granules to have the same constitution as the 'epiplasts' of vegetative cells, that is, having a resistant outer layer of 'nucleo-glyco-protein' and a more proteinaceous interior. This corresponds to a certain extent with the finding that arginine and Feulgen-staining material are sometimes both concentrated at the poles. Baumgärtel's observation (1920) that the outer nucleotide-containing layer of a polar granule may sometimes be absent also finds a parallel in the results obtained with *Anabaena cylindrica*. The problem of the nature and significance of polar granules cannot yet be regarded as satisfactorily settled, but this uncertainty does not affect the conclusions regarding the depletion of heterocysts set forward in this paper.

It is remarkable that nothing resembling the transverse zonation found in *Anabaena cylindrica* has been recorded by previous workers. There can be no doubt about the existence of this feature since it can be demonstrated by various independent methods and because the possibility that it is an artefact is ruled out by its being visible in living unstained material under critical illumination. It does not appear to be peculiar to *Anabaena cylindrica* since clear examples have been found in haematoxylin preparations of the taxonomically widely separated form, *Hapalosiphon* sp., grown in laboratory culture.

Many workers have stated that old heterocysts contain vacuoles, and Bharadwaja (1933) has recorded vacuolation beginning before cell extension was complete in *Aulosira fritschii*. Although the protoplast of a heterocyst of *Anabaena cylindrica* can have a low content of dry matter, as is shown by the marked shrinkage on drying, no vacuoles have been observed in the course of this investigation. This may perhaps be due to the conditions under which the alga was grown.

While there is no evidence for the existence of plasmodesmata of diameter greater than 0·1 μ between normal cells, it is not clear whether during the formation of a heterocyst the septum disappears so that protoplasmic connexion is established with adjacent cells. The connexions of a heterocyst stain with haematoxylin and other reagents in the same way as its protoplast and have been regarded by Geitler (1921) as plasmodesmata, but it is possible that it is the inner investment which takes up the stain and that the lumen of the connexion is occupied by non-living material (see Fritsch, 1945, p. 791). The resolution of the visual light microscope is insufficient to enable this point to be decided and the ultra-violet photomicrographs also offer only equivocal evidence.

Geitler (1921) has recorded germination of heterocysts in many species of blue-green algae and this phenomenon has also been observed in *Anabaena cylindrica* (Fogg, 1949). Geitler states that it is the fully developed structures which do this, whereas heterocysts that have not yet formed a cellulose wall do not possess the capacity, so that it appears that germination is still possible after the protoplast has become homogeneous and the structures present in the centroplasm have disappeared. It is not known if the capacity to germinate is retained after depletion of a heterocyst has begun.

The belief that heterocysts are the seat of salt accumulation (Fogg, 1944) has been shown here to be erroneous and the suggested resemblance between the formation of these structures and vacuolation in plants other than blue-green algae now appears superficial. Evidence has been presented in another paper (Fogg, 1949) showing that the formation of a heterocyst from a normal cell begins when some specific nitrogenous substance within it, probably ammonia, falls below a critical concentration. This event seems to initiate a fundamental change in the organization of the protoplast so that previous metabolic trends are altered, polysaccharide synthesis being now increased, whereas nitrogenous cell structures are broken down. The mobilized material appears to be transferred to adjacent cells. A possible biological advantage of heterocysts may thus be that their formation, which is more frequent the lower the general level of the nitrogen nutrition of the alga, results in the release of materials which may be used to maintain adjacent cells. This idea is not incompatible with that of Serpette (1948), who suggests that heterocysts serve certain important mechanical functions.

ACKNOWLEDGEMENTS

I am deeply grateful to Professor Irene Manton for her generous help with the ultra-violet photomicrography and for her criticism of this paper in its manuscript stages.

My thanks are also due to Mr. R. Brinsden and Miss D. Cuthbert for assistance with photography and microscopical preparations respectively.

LITERATURE CITED

BAKER, J. R., 1947: The Histochemical Recognition of Certain Guanidine Derivatives. Quart. J. micr. Sci., lxxxviii. 115–21.

BARNARD, J. E., and WELCH, F. V., 1936: Practical Photomicrography. London, Edward Arnold and Co., 3rd ed.

BAUMGÄRTEL, O., 1920: Das Problem der Cyanophyzeenzelle. Arch. Protistenk., xli. 50–148.

BHARADWAJA, Y., 1933: Contributions to our Knowledge of the Myxophyceae of India. Ann. Bot., xlvii. 117–43.

BOURNE, G. (ed.), 1942: Cytology and Cell Physiology. Clarendon Press, Oxford.

CASPERSSON, T., 1947: The Relations between Nucleic Acid and Protein Synthesis. Symp. Soc. Exp. Biol., i. 127–51.

FOGG, G. E., 1944: Growth and Heterocyst Production in *Anabaena cylindrica* Lemm. New Phytol., xliii. 164–75.

—— 1949: Growth and Heterocyst Production in *Anabaena cylindrica* Lemm. II. In Relation to Carbon and Nitrogen Metabolism. Ann. Bot., N. S., xiii. 241–59.

FRITSCH, F. E., 1945: The Structure and Reproduction of the Algae, vol. ii. Cambridge University Press.

GEITLER, L., 1921: Versuch einer Lösung des Heterocysten-Problems, Sitzber. Akad. Wiss. Wien, Mat.-Nat. Kl. 1, cxxx. 223–45.

JOHANSEN, D. A., 1940: Plant Microtechnique. McGraw-Hill Book Company Inc., New York.

MAZUR, A., and CLARKE, H. T., 1938: The Amino Acids of Certain Marine Algae. J. Biol. Chem., cxxiii. 729–40.

—— —— 1942: Chemical Components of Some Autotrophic Organisms. Ibid., cxliii. 39–40.

MÜHLDORF, A., 1938: Einige Betrachtungen zur Membranmorphologie der Blaualgen. Ber. Deutsch. Bot. Ges., lvi. 316–36.

POLJANSKY, G., and PETRUSCHEWSKY, G., 1929: Zur Frage über die Struktur der Cyanophyceenzelle. Arch. Protistenk., lxvii. 11–45.

SERPETTE, M., 1948: Morphologie de *Homeothrix balearica* (Born. et Fl.) Lemm. et fonction des hétérocystes chez les Algues bleues. Bull. Soc. bot. Fr. xcv. 295–300.

SPEARING, J. K., 1937: Cytological Studies of the Myxophyceae. Arch. Protistenk., lxxxix. 209–78.

ULLRICH, H., 1930: Über die Bewegungen der Beggiatoaceen und Oscillatoriaceen. II. Mitteilung. Planta, ix. 144–94.

EXPLANATION OF PLATES

Illustrating G. E. Fogg's article on 'Growth and Heterocyst Production in *Anabaena cylindrica* Lemm. III. The Cytology of Heterocysts'.

Figs. 1, 3, 4, 5, 6, 7, 9, 10, 11. Ultra-violet photomicrographs of *Anabaena cylindrica* taken at 2,750 Å. Condenser aperture 6 mm.; exposure 5–10 sec.; final magnification 3,000 diameters. For other details see text.

Figs. 2, 8. Photomicrographs of Heidenhain haematoxylin preparations of *Anabaena cylindrica*. Orange filter (Wratten E); Koristka immersion semi-apochromatic objective, 0·17 mm., N.A. 1·30; × 10 compensating ocular; final magnification 1,500 diameters.

These examples came from the following samples of the culture series described on p. 23: Pl. II, Figs. 1 and 2, sample 0; Fig. 3, sample 1; Fig. 4, sample 2; Fig. 5, sample 4; Fig. 6, sample 8. Pl. III, Figs. 7, 8, and 9, sample 8; Fig. 10, sample 23; Fig. 11, sample 38.

Figs. 12, 14. Dried preparations of *Anabaena cylindrica* mounted in absolute alcohol. Photomicrographical details as for Figs. 2 and 8. For other details see text, p. 25.

Figs. 13, 15. The filaments illustrated in Figs. 12 and 14 after micro-incineration. The preparations were mounted dry, details otherwise as for Figs. 12 and 14.

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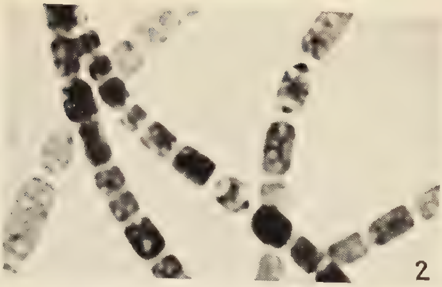
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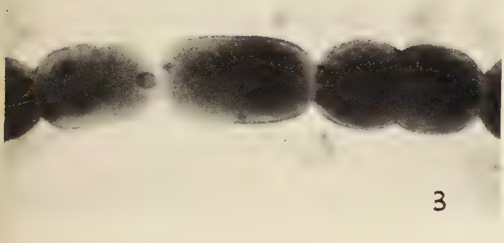
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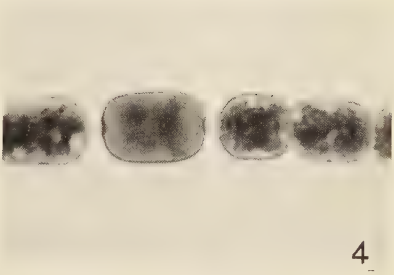
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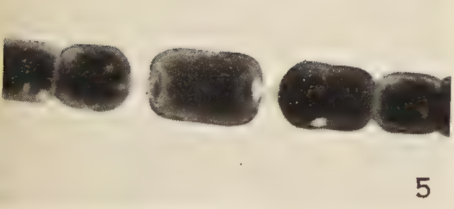
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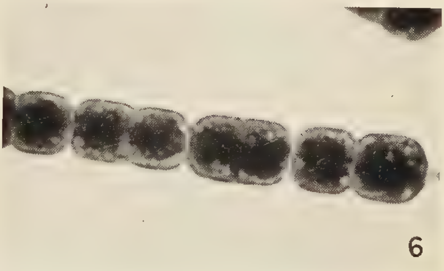
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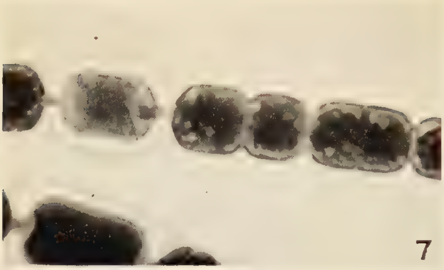
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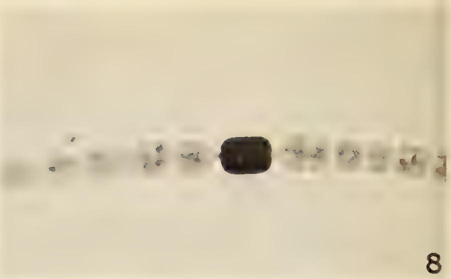
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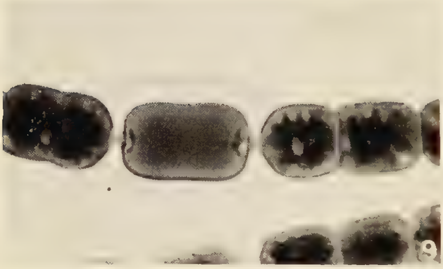
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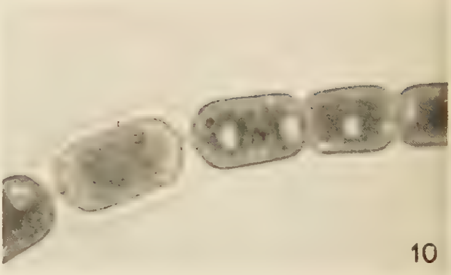
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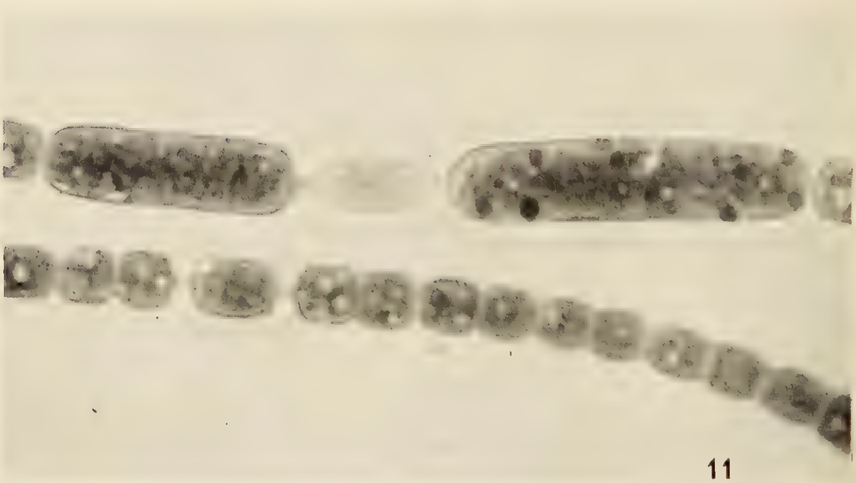
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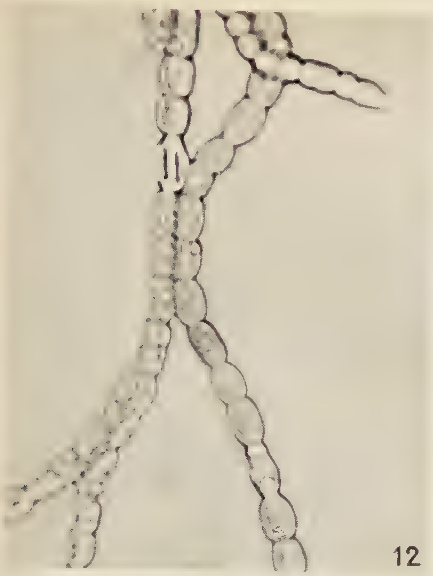
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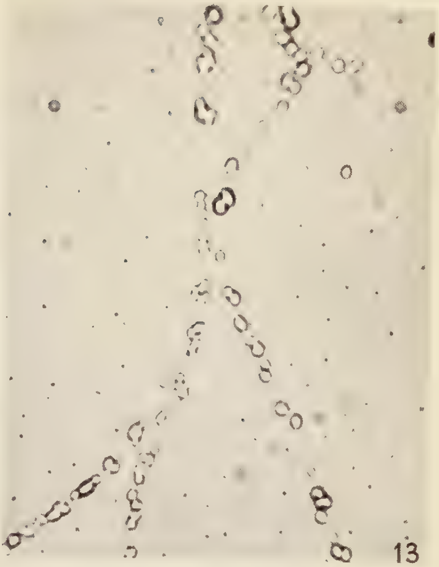
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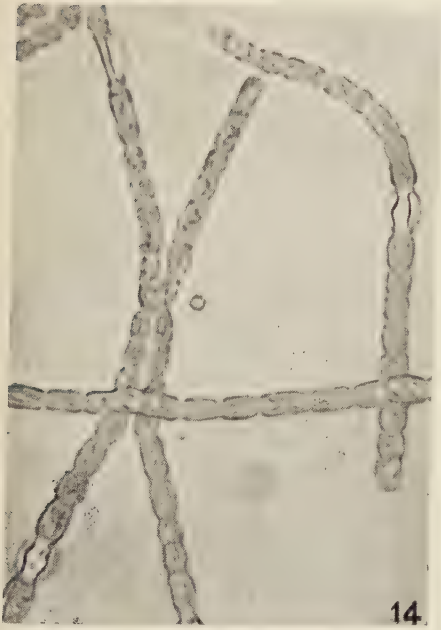
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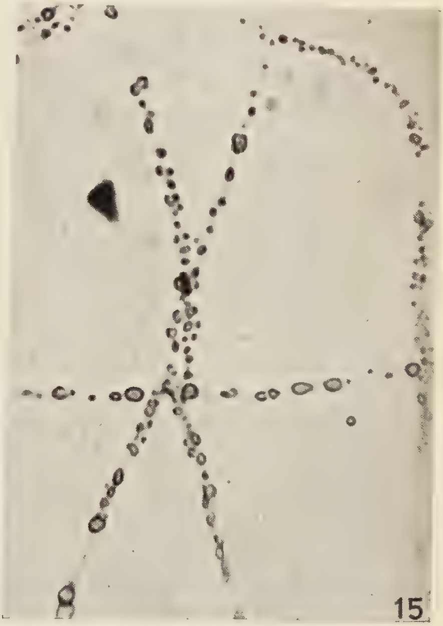
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G. E. FOGG

The Anatomy of *Stenochlaena palustris* (Burm.) Bedd.

BY

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(*Punjab University, Amritsar (India)*)

With Plate V and twenty-four Figures in the Text

ABSTRACT

The stelar anatomy of *Stenochlaena palustris* is described. The stem is dictyostelic and in transverse section shows five or six closely situated large central meristeles. Around these are two rings of smaller peripheral bundles. These outer rings are mainly 'residual traces' derived from the leaf- and root-traces. They anastomose freely and are intimately connected with the central system.

The dictyostely of the latter is due both to leaf-gaps and to more or less random perforations, the latter being the more numerous.

The leaf-trace, at first binary, enters the petiole along with a large number of the smaller bundles from the stem periphery. 'Root-traces' are given off from the base of the leaf-gaps.

THE systematic position of the genus *Stenochlaena* is dubious. Holttum (1932) and Christensen (1938) have placed it in the Pterideae, while Bower (1928) and Copeland (1947) consider its proper place in the Blecknoideae.

The members of the genus possess a climbing habit and are found in the tropics. They are characterized by a great variability in the form of leaves which in some cases serve to attach the plant to its support.

Stenochlaena palustris (Burm.) Bedd. was collected from Sikkim at an altitude of about 800 ft. near Tista Bridge, *en route* to Gangtok, where the trunks of forest trees are densely covered with this fern climber. In a tract of the forest about 5 miles on either side of the motor road the species is observed on almost all the trees arising nearly to the tree-tops (Pl. V, Fig. 1). This is done with the help of an elongated scandent branched rhizome which anchors itself to the bark of trunks by means of sticking adventitious roots. These latter arise mostly in groups all along the stem, but here and there single roots may also be given off. The side of the stem which is adpressed to the trunk is somewhat flattened and the roots are chiefly located on this side. The stem possesses long internodes. The lower leaves are all sterile, but in the upper region the fertile ones are observed. The leaves are 1 to 4 ft. long, pinnate, and of hard texture. The sterile pinnae are sharply serrate, while the fertile ones have an entire margin which is somewhat reflexed. In the sterile pinnae the veinlets form areolae on either side of the costa, while elsewhere they are free.

[Annals of Botany, N.S. Vol. XV, No. 57, January, 1951.]

The material for the present investigation was fixed in formalin-acetic-alcohol. Serial hand sections were cut and stained with a combination of safranin and light green.

STEM ANATOMY

A transverse section through an internode of the rhizome is almost oval in outline with the side towards the support somewhat less convex than the other. The cells of the epidermis are small and tabular in outline (Text-fig. 2). Immediately below the epidermis is a band of sclerenchyma consisting of several rows of cells. These cells develop thick brown walls, but retain protoplasmic contents in their small round cavities. This sclerenchymatous layer is sharply delimited from the thin-walled cortex underneath. The cells of the cortex are large and polygonal. The cortex communicates with the pith through the narrow gaps in the main stelar system. The pith is small and its cells are of the same nature as those of the cortex.

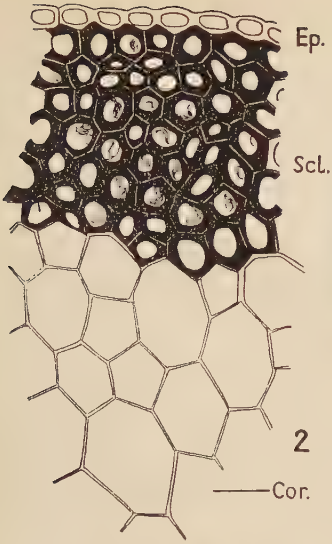
In section the stem shows a central vascular system of large meristeles surrounded by a peripheral one of small bundles embedded in the cortex. The five or six large central meristeles form a ring round the pith (Text-fig. 1 and Pl. V, Fig. 2). The large size of the meristeles and the narrow gaps are in accordance with the climbing habit of the species. The smaller bundles of the peripheral system form two concentric rings in the cortex, of which the inner one is usually incomplete on the side of the support. The bundles of the inner ring are smaller than those of the central system, while those of the outer ring are smaller still.

Each meristele of the central system is surrounded by a thick sclerenchymatous sheath which delimits it from the surrounding tissue (Pl. V, Figs. 2, 3). Next comes a complete endodermal ring whose cells possess the usual Casparian thickenings (Fig. 3). Within it is the pericycle one or two layers of cells in thickness, the cells containing brown particles. The phloem tissue consists of large sieve tubes, polygonal in outline in transverse section, and thin-walled small-celled conjunctive parenchyma. The phloem surrounds the xylem on all sides excepting on the two lateral ends. The main bulk of the meristele is formed of large metaxylem elements. On the two lateral sides are found two protoxylem groups abutting directly on the pericycle.

The bundles in the peripheral stelar system have the same structure as described above, except that there is only one protoxylem group.

The longitudinal appearance of the larger meristeles is shown in Text-fig. 4.

The opening of a gap in the central stele is always initiated by the giving off of a small basal strand to the periphery. This is designated the 'root-trace', as in the allied species *S. sorbifolia* it directly passes into the adventitious root (Bower, 1914). The course of this 'root-trace' in the present species, however, is not so simple and is traced in a series of transverse sections of the stem represented in Text-figs. 5–12. In Text-fig. 5 the bundle *A* is observed giving off a small 'root-trace' *C* from the centre where it opens to form a



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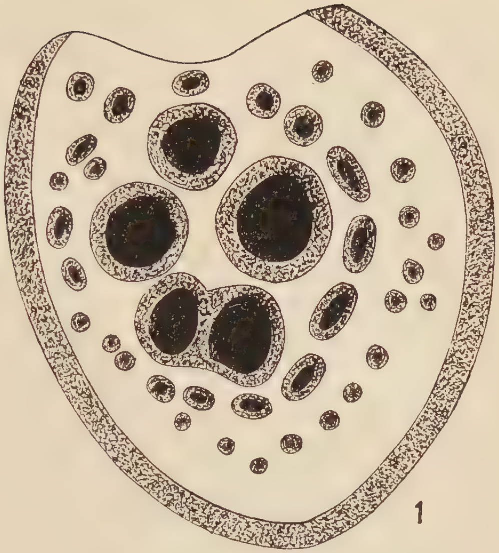

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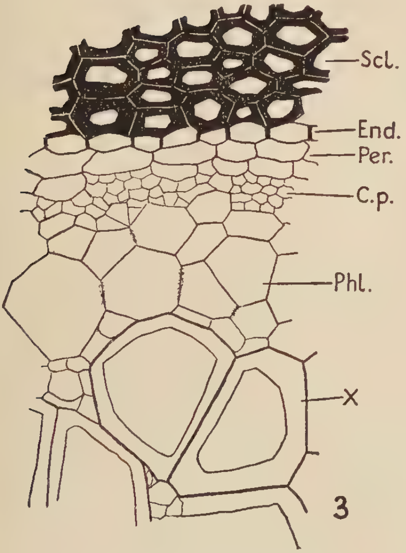

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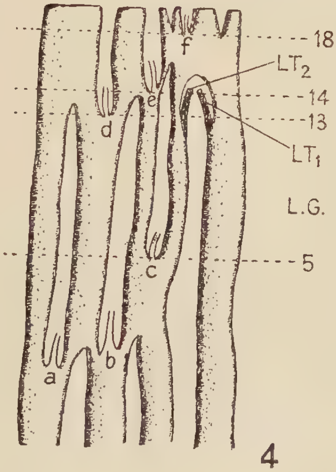

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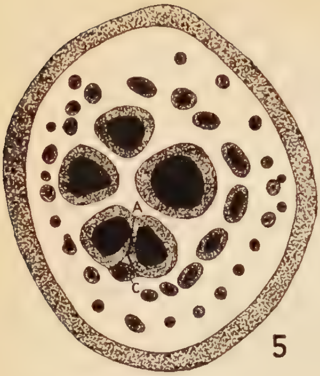
Fig. 1. T.S. through the internode of *Stenochlaena palustris.* ×15.
Fig. 2. T.S. through the cortex. ×216.
Fig. 3. T.S. of a portion of the meristele. ×216.
Fig. 4. Diagrammatic representation of the central stelar system flattened in one plane as reconstructed from serial sections shown in Figs. 5–19. The dotted region surrounding each bundle represents the sclerenchymatous envelope.

Ep. Epidermis, Scl. Sclerenchyma, Cor. Cortex, End. Endodermis, Per. Pericycle, C.p. Conjunctive parenchyma, Phl. Phloem, X Xylem. In Fig. 4 *a–f* represent basal strands which initiate the perforations. L.G. is leaf-gap. LT_1, LT_2 binary leaf-trace given out just before the leaf-gap closes.



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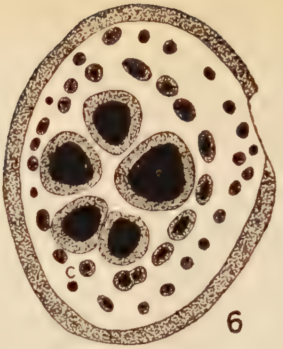

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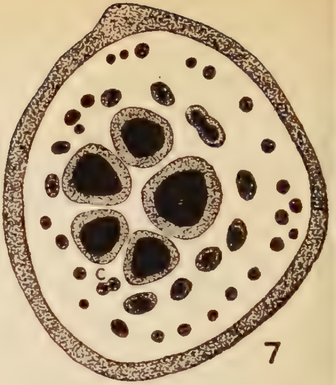

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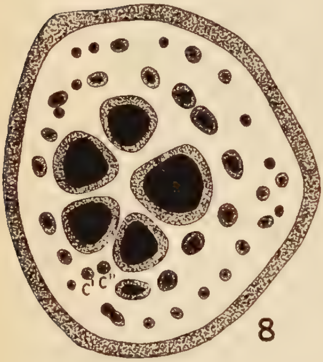

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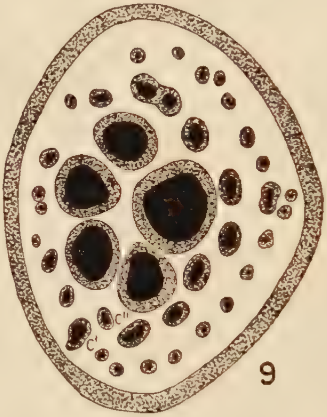

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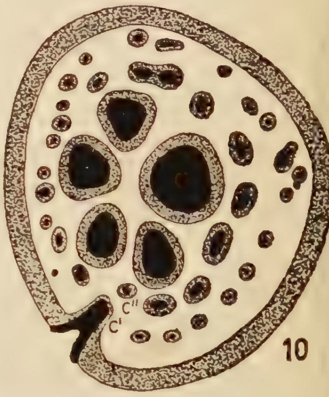

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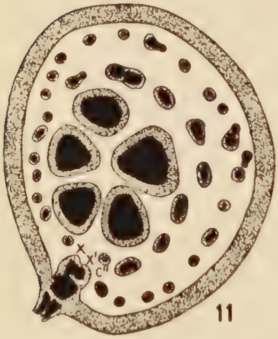

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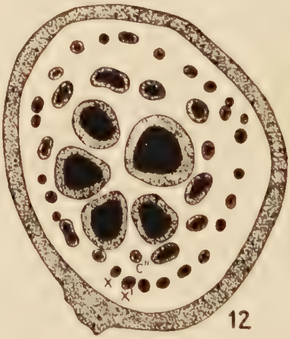

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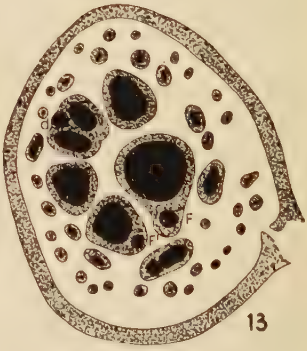

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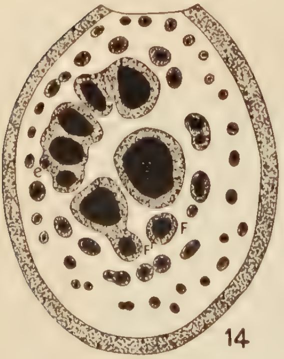

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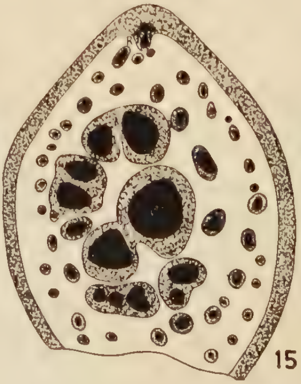

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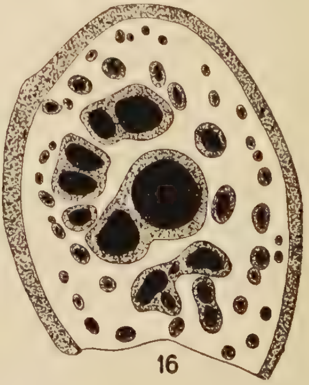

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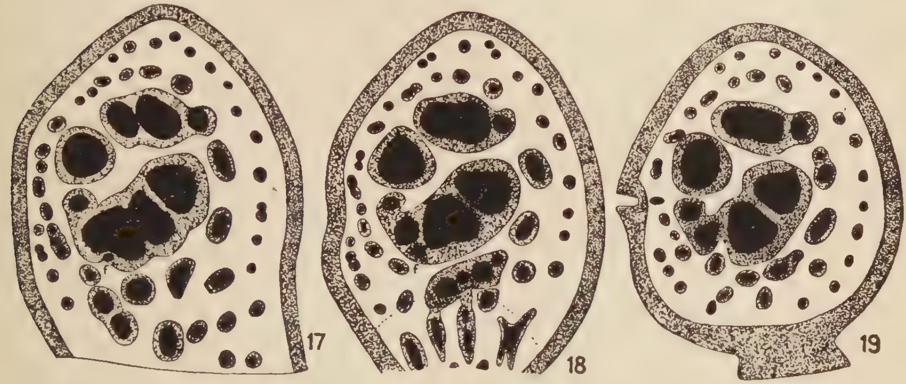
gap (Text-fig. 6). This bundle as it passes into the cortex soon divides into two bundles C' and C'' (Text-figs. 7, 8). The bundle C'' forms a part of the peripheral system. The bundle C' anastomoses with one of the peripheral bundles (Text-fig. 9) and then proceeds to give off traces to a bunch of roots formed on this side of the stem after leaving two residual strands X and X' in the peripheral system (Text-figs. 11, 12). Thus the original 'root-trace' bundle given off from the primary stele at the opening of the gap not only forms 'root-traces' but also leaves a few small strands in the cortex forming the peripheral system. The gap continues for a fairly long distance and then closes without any further disturbance. These gaps in the central system are, therefore, merely of the nature of perforations and they appear and disappear frequently.



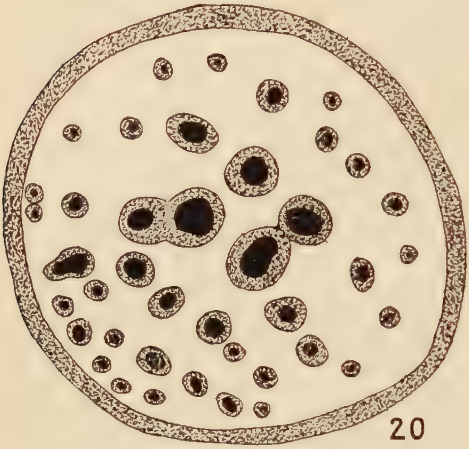
FIGS. 5–19. Serial sections of stem of *S. palustris* from below upwards illustrating:
(*a*) the emergence of 'root-trace' C and its further course (Figs. 5–12) and
(*b*) the binary leaf-trace and its fate in the cortex (Figs. 13–19). × 10.

Text-fig. 4 gives a diagrammatic representation of the central stelar system flattened in a single plane and shows these perforations reconstructed from a series of transverse sections. The 'root-traces' at the base of the meshes are marked *a*, *b*, *c*, *d*, *e*, and *f*. The marks 5, 13, 14, and 18 correspond to the transverse sections at these regions shown in the figures bearing the same numbers.

The vascular strands of the peripheral system go on fusing and dividing without any regularity, forming a highly reticulate network surrounding the central stele. As described above there are two such networks surrounding the main stele on the outer side of the stem. Some of these strands may pass off as traces to the solitary roots present here and there on the stem (Fig. 15).

The leaf-trace

The fronds in this species are placed so far apart that the leaf-traces departing to two successive leaves are never observed in the same transverse section. The leaf-gap arises in the same manner as an ordinary perforation described above, i.e. by the giving off of a 'root-trace', but is differentiated from a perforation by the fact that just before it closes two leaf-trace bundles are given off, one from each of the two meristeles enclosing the gap (Text-fig. 4; L.G. is leaf-gap and LT_1, LT_2 leaf-trace bundles). The initiation of the two leaf-trace bundles *F* and *F′* is shown in Text-fig. 13. In Text-figs. 14–18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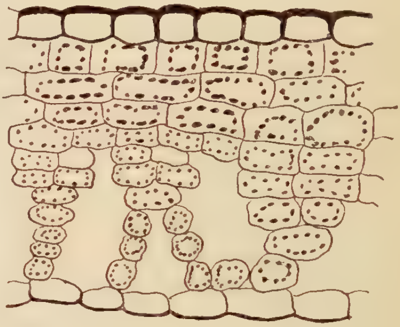
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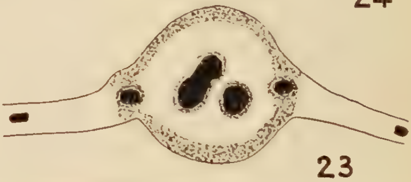
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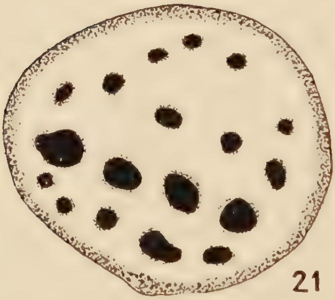
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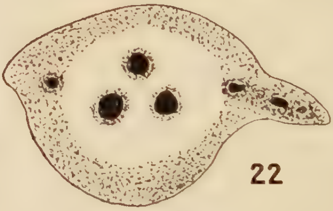
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FIG. 20. T.S. through petiole. Mark unequal size and irregular distribution of vascular bundles. × 15.

FIG. 21. T.S. through rachis. × 15.

FIG. 22. T.S. through the small stalk of a pinna. × 15.

FIG. 23. T.S. through the mid-rib of a pinna. × 15.

FIG. 24. T.S. through a pinna. Note the elongated palisade cells parallel to the leaf-surface. × 385.

the course of these strands as they pass through the cortex to the base of the petiole is shown. The two leaf-trace bundles anastomose without any regularity with some of the vascular strands of the peripheral system present in the cortex (Text-figs. 15, 16). These, together with some other peripheral strands in the cortex on this side, pass off into the petiole, leaving a few residual strands in the cortex. Text-fig. 18, representing a transverse section through the node, shows that a lot of division and fusion has taken place at the base of the petiole, and as a result thereof the leaf-trace at this stage consists of a large number of strands disposed without any regularity.

PHYLLOPODIUM

The petiole is thick and long. In transverse section it is almost circular in outline (Text-fig. 20). A complete ring of sclerotic tissue is present below the epidermis. A large number of vascular strands are irregularly scattered in the ground parenchyma which is formed of thin-walled polygonal cells. The large bundles in the centre show signs of fusion. The structure of each vascular bundle is the same as that of the meristele of the stem except that the protoxylem is found in two or three groups on one side of the metaxylem.

The transverse section of the rachis shows that the irregular arrangement of the vascular strands in the petiole is continued upwards but the number of these becomes reduced (Text-fig. 21). The hypodermal layer of sclerenchyma is present, but the sclerenchymatous sheath around the bundles is not much developed, only the walls of the cells adjacent to the endodermis being thickened.

The vascular supply to the short stalk of the pinna consists of three strands (Text-fig. 22). As these pass upwards into the mid-rib they fuse to form two (Text-fig. 23). Small traces are given off laterally from these big strands to supply the lamina. Both the strands of the mid-rib as well as the traces given to the lamina are surrounded by a sclerenchymatous sheath.

The ground tissue of the mid-rib shows much the same structure as that of the petiole. Several layers of hypodermal sclerenchyma cells are also present.

In the lamina the cells of the mesophyll are differentiated into palisade and spongy tissue, but the elongated cells of the palisade lie parallel to the leaf-surface instead of being at right angles to it. Both the upper and the lower epidermis possess cuticle which is more thickly developed on the former than on the latter.

DISCUSSION AND CONCLUSIONS

The vascular anatomy of only two species of *Stenochlaena*, namely, *S. tenuifolia* and *S. sorbifolia*, has so far been published.

According to Mettenius the axis of *S. tenuifolia* is traversed by a dictyostele, which in transverse section consists of five or six meristeles each surrounded by a complete endodermal sheath (Bower, 1923). The disintegration of the stele takes place by simple perforations as well as by a smaller number of leaf-gaps as seen in the diagram of the stelar system given by the author. A basal strand or 'root-trace' is not observed to be given off at the base of a perforation or a leaf-gap. No further details are given.

Anatomy of *S. sorbifolia* was investigated by Bower (1914), who states that the vascular system in the axis is a modification of the *Blechnum* type. There are five or six large meristeles arranged in a ring in the centre with very narrow leaf-gaps at the base of each of which arises a 'root-trace'. He does not make any mention of perforations nor of the presence of a peripheral stelar system. The proportion of xylem in the meristele is quite large in accordance with the climbing habit of the species. More than one leaf-trace is present in a single transverse section. The leaf-trace is composed of four to six *bundles arranged in the form of a horse-shoe.*

The anatomy of *S. palustris* is much more complex than that of either of the above two species. There is a cortical system of strands besides the central one. The latter is formed of five or six large meristeles with very narrow spaces in between. The central stele is disintegrated by perforations as well as leaf-gaps. The former are far greater in number than the latter. They are narrow and elongated, and arise by the separation of a basal strand which does not behave directly as a 'root-trace', as is the case in *S. sorbifolia*, but during its course outwards in the cortex leaves a few residual strands and then supplies the adventitious roots which arise on the stem surface. The leaf-gaps are fewer and arise in the same fashion as the perforations by giving out a 'root-trace', but higher up, just before closing, give off a binary leaf-trace. Unless a study of the serial sections is made it is not possible to differentiate between perforations and leaf-gaps. In fact perforations are like leaf-gaps minus the leaf-traces.

The cortical system is built up from the branches of the 'root-traces' as they pass out into the cortex after emerging from the bases of perforations or leaf-gaps and is thus connected with the central system. These cortical strands anastomose variously and form a reticulate system which may be in the form of one or two concentric envelopes around the central stele.

The two leaf-trace strands on their passage into the cortex fuse with the cortical strands in all possible manners. Ultimately the leaf-trace, which receives a considerable supply of vascular bundles from the peripheral system also, consists of a large number of strands arranged irregularly and in this manner passes out into the petiole.

It is evident that the stelar system in *S. palustris* is built on a much more elaborate plan than that in *S. tenuifolia* or *S. sorbifolia*. This complexity seems to be related to the physiological needs of the species in the matter of conduction of water and food supply. The stem is highly elongated, rising to tree-tops, and bears a large number of big fronds. The stem relative to its length is rather thin and with insufficient development of the vascular tissue in the primary system. Secondly secondary growth is absent as in the entire fern relationship. The species has solved the problem by the development of a peripheral system of vascular strands in the cortex.

It is also evident that the leaf-trace in the present species as it passes into the petiole becomes much more complex than the relatively simpler leaf-trace present in *S. sorbifolia*, which consists of five or six strands arranged in the form of a horse-shoe.

Phyletic affinity of the genus

In the words of Professor Bower (1928), the genus *Stenochlaena* 'has suffered great vicissitudes of nomenclature'. It has been referred to *Lomaria* by Wilderow, to *Onoclea* by Swartz, and to *Acrostichum* by Baker. Holttum

(1932) and Christensen (1938) have considered the genus to be related to *Acrostichum* and have placed it in Pterideae. Smith originally suggested the affinity of this genus with the Blechnoids, and Bower (1928) has supported this view partly on the basis of the pinnate nature of the fronds and their hard texture, partly because of the dictyostelic nature of the axis stele with its narrow leaf-gaps. These give off a 'root-trace' at the base. And this and the binary leaf-trace given off from the axis stele are features which are in common with the Blechnoids. The genus is regarded as closely related to the *Lomaria* section of the comprehensive genus *Blechnum* because of the dimorphic leaves and the sporangial region protected by involuted leaf-margin as well as the non-development of the flange, but differs in the development of an acrostichoid state which is considered to be a derivative feature and an advance over *Lomaria*. In a recent work Copeland (1947) lends further support to this relationship on the basis of the spore structure, which is bilateral and therefore different from the tetrahedral spore met with in Pterideae.

From anatomical evidence the genus *Stenochlaena* certainly shows affinity with the Blechnoids. In the species investigated the gap in the axis stele invariably starts after giving off a 'root-trace' at the base, although in *S. palustris* the latter may become further modified during its course through the cortex. Also there are originally two leaf-trace strands departing from the central stele as in Blechnoids. In Pteridae, on the other hand, a leaf-gap does not start with a 'root-trace' at the base, and secondly the formation of a binary leaf-trace is not a characteristic feature of this family; more often the leaf-trace is simple and strap-shaped.

It must, however, be emphasized that in none of the Blechnoids is a vascular system as complex as that of *S. palustris* met with in the axis or in the phyllopodium. The origin of this seems to be an adaptation to the physiological needs of the species. The cortical reticulate system owes its origin to the modifications in the 'root-traces' during their passage through the cortex, necessary to meet the exigencies of conduction in this climbing species with elaborate vegetative development.

LITERATURE CITED

Bower, F. O., 1914: Studies in the Phylogeny of Filicales. Ann. Bot., xxviii.
—— 1923, 1928: Filicales, vols. i, iii. Cambridge.
Christensen, C., 1938: Manual of Pteridology.
Copeland, E. B., 1947: Genera Filicum, Waltham, Mass., U.S.A.
Holttum, R. E., 1932: Garden's Bull. 5.

EXPLANATION OF PLATE V

Illustrating P. N. Mehra's and N. Chopra's article on 'The Anatomy of *Stenochlaena palustris* (Burm.) Bedd.'

Fig. 1. *Stenochlaena palustris* ascending to the tree-tops in Tista Valley, Darjeeling District.
Fig. 2. Transverse section through the internode of *S. palustris*.
Fig. 3. A meristele of the central stelar system.

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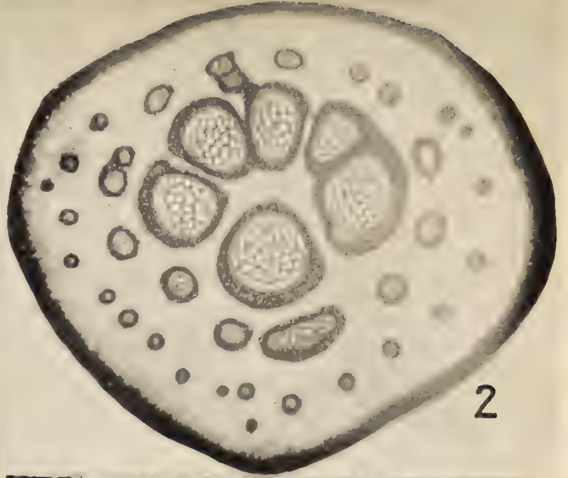
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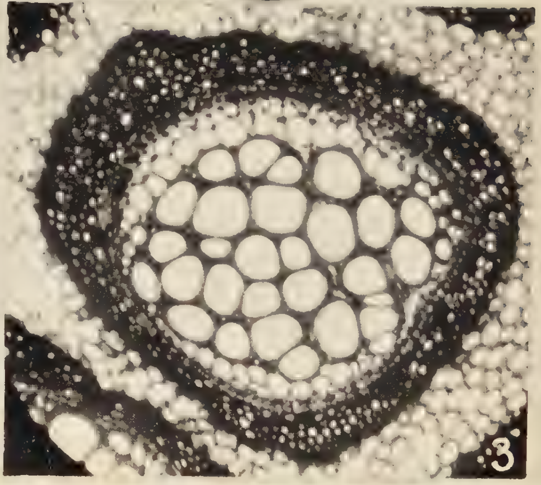
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P. N. MEHRA and N. CHOPRA

The Effects of the Gamma Isomer of Benzene Hexachloride (Hexachlorocyclohexane) on Plant Growth and on Mitosis

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ABSTRACT

Three experiments have been carried out on the effects of the gamma isomer of benzene hexachloride (hexachlorocyclohexane), applied in the form of a china clay dust containing appropriate amounts of the pure compound.

The first experiment showed that root-growth of cress was unaffected by gamma-BHC, but that in wheat, growth was significantly reduced.

The effect of the compound on mitosis in rye was shown by the second experiment to resemble that of colchicine and other mitotic poisons in inducing polycentric mitoses or polyploidy by inhibiting spindle action and in causing enlarged nuclei of irregular shape which contained increased amounts of nucleolar material.

In the third experiment the threshold concentrations for root-growth inhibition and for the production of abnormal cytological effects were found to be the same.

It is considered that the growth-regulatory and mitotic effects of gamma-BHC are causally related.

INTRODUCTION

THE insecticidal properties of the gamma isomer of benzene hexachloride, the active principle of 'Gammexane', have now become well known. From time to time, however, direct effects on plant growth in field trials have been noticed when this compound was applied as a seed dressing. In some cases when high concentrations were used stunting was caused, while in relatively few others at lower strengths growth appeared to have been stimulated. Where stunting occurred the young plants had swollen root-tips and, in the case of cereals, short, thickened, strap-like leaves, symptoms which were superficially similar to those induced by appropriate concentrations of isopropylphenylcarbamate (IPPC) and 2-methyl-4-chlorophenoxyacetic acid ('Methoxone', MCPA), of which the former was already known to cause mitotic abnormalities (Doxey, 1949).

The John Innes Horticultural Institution report for 1945 made a brief reference to the cytological effect, stating that 'the pest control drugs DDT and "Gammexane" differ in their action on plant nuclei. While "Gammexane"

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induces polyploidy by suppressing the metaphase spindle, DDT has no such action.'

It was therefore considered that the growth-regulating and cytological effects of the gamma isomer of benzene hexachloride might be causally related and, with this in view, experiments were carried out at Jealott's Hill during 1947–9. The results of these experiments are given in the present paper. The abbreviation 'gamma-BHC' is used throughout this publication for the pure gamma isomer following the recommendation of the B.S.I. Insecticide Nomenclature Committee.

In 1948 D. Kostoff reported that the gamma isomer interfered with 'the cytoplasmic processes involved in the formation of the achromatic figure', causing repeated chromosome doubling, enlarged nuclei and cells, and occasionally the formation of multinucleate cells or deformed nuclei. He also reported that chromosome or chromatid fragmentation was found on occasion.

Quidet and Hitier (1948) also reported that 8 per cent. dusts of 'hexachlorocyclohexane' (the isomer used was not specified) caused anomalies of growth, and that the anomalous plants were polyploid.

EXPERIMENTAL DETAILS

In our early experiments measurements of root growth were made on wheat and cress, while rye was used for the cytological work, the same gamma-BHC treatments being used for both sets of investigations. These species were selected as being the most suitable for the purposes intended. Later, measurements of root growth and cytological observations were combined in a single experiment on wheat. Rye proved unsuitable for root-growth determination owing to low and irregular germination rate.

In all experiments treatments were applied as dusts of gamma-BHC in china clay, prepared by dissolving a given weight of the pure gamma isomer in acetone, adding a given amount of china clay, stirring, evaporating off the acetone at room temperature in a stream of air, and finally thoroughly shaking the resultant dusts.

In this manner seven treatment dusts were prepared, containing 0 (control), 0·1, 1, 5, 10, 20, and 30 per cent. of pure gamma-BHC.

Experiment I. Effect of gamma-BHC on root growth of cress and wheat

Seeds of cress (*Lepidium sativum*) and of wheat (*Triticum vulgare*, var. 'Atle') were dressed with a given dust at the rate of 2 oz. per bushel of seeds. For each species, 10 seeds from a given treatment were germinated in the dark at a constant temperature of $70 \pm 2°$ F., on an agar medium (pH 7) contained in a Petri dish, the agar surface being placed perpendicular to the bench top and the line of seeds parallel with it. There were six replicates of each treatment.

The root lengths of cress were measured after 4 days while the wheat measurements were made at the end of 5 days.

Root-growth measurements on cress and wheat are given in Table I.

TABLE I

% gamma isomer in dust	Treatment mean root length per seed (mm.) Cress	Wheat
0	24·0	42·4
0·1	26·1	56·5
1	21·8	52·8
5	29·4	53·3
10	29·6	35·5
20	26·5	26·3
30	28·8	21·8
S.E. per treatment (as % of mean value of all plates)	10·2%	10·7%
Sig. diff. between treatments ($P = 0{\cdot}05$)	7·7	12·6

Thus it is seen that gamma-BHC had no effect on the root growth of cress seedlings even at the highest treatment level (30 per cent. dust). With wheat the 0·1, 1, and 5 per cent. dust treatments showed, on the average, an increase in root growth over the control, but only in the case of the 0·1 per cent. dust was the increment significant. At the higher rates of treatment the reverse effect was observed, there being a significant reduction in the root growth of seed which had been dressed with dusts containing 20 and 30 per cent. of the gamma isomer.

Experiment II. Cytological effects induced in rye

Seeds of rye (*Secale cereale*) were treated with china clay dusts containing 0 (control), 0·5, 10, 20, and 30 per cent. pure gamma-BHC in a similar manner to that already described for the experiment on seedling growth, the rate of dusting again being 2 oz. per bushel.

After dusting, seeds from each treatment were sown under separate Copenhagen jars on filter card kept moist with distilled water. The seeds were germinated in the dark at a temperature of 70±2° F.

When the seedlings had developed roots of about 1 cm. in length (approx. 65 hours after sowing), the root-tips were removed and fixed in a chromacetic fixative (1 per cent. chromic acid, 9 vols., to 5 per cent. acetic acid, 1 vol.) for 1 hour. After washing for 1 hour in six changes of water at 35–40° C., preparations were made by the Feulgen technique. Hydrolysis in N.HCl at 60° C. for 20 minutes was followed by staining in leucobasic fuchsin (prepared by Coleman's method) for 1 hour. Root-tips were then washed in SO_2 solution for half an hour and squashed under the coverslip. Preparations were made permanent by dehydrating and mounting in Canada balsam in xylene. Photomicrographs of typical preparations were made and a study of these, combined with direct microscopic examination, led to the observations described below.

Treatment with dusts containing 0·5 per cent. of pure gamma-BHC had no visible effect on mitosis. The higher rates of treatment, however, all caused serious mitotic disturbances, the metaphase spindle being suppressed. In consequence, chromosomes at metaphase were not arranged on the equatorial plate, but lay scattered (often closely paired) throughout the cell, in a disorderly manner. Polyploid nuclei were found, their frequency and degree of polyploidy increasing with increasing concentration of the compound.

Interphase nuclei were enlarged and frequently highly irregular in outline, being lobed or consisting of several bodies connected by fibrils.

Following treatment with 10 per cent. dusts, polycentric mitoses ('multipolar spindles') were common. They occurred much less frequently after treatment with 20 per cent. dusts and were absent at the 30 per cent. treatment level.

Nucleoli in roots treated at 10 per cent. and above were enlarged or multiplied, the increase in nucleolar material in the resting nuclei apparently following approximately the increase in polyploidy. There appeared to be no evidence of chromosome or chromatid breakage, or of disturbed nucleic acid metabolism.

The rate of mitosis was obviously reduced by all except the 0·5 per cent. treatment. The number of visible abnormal metaphases was high after treatment with 10, 20, and 30 per cent. dusts; prophases became rarer with increasing concentration and anaphases were absent at the 20 and 30 per cent. levels. Normal mitoses were interspersed with abnormal after treatment at 10 per cent. but were absent at higher levels.

Experiment III. Effects on root growth and mitosis in wheat

Seeds of wheat (*Triticum vulgare*, var. 'Holdfast') were treated and sown as before, except that a dusting rate of 4 oz. per bushel was used. Similar cytological methods were followed, except that a hydrolysis time of 25 minutes proved necessary for this species, and a counterstain (fast green FCF, approx. 1 per cent. in 95 per cent. alcohol) was applied before making the preparations permanent. The measurements of root length are given in Table II.

TABLE II

Effect of gamma-BHC on the Root Growth of Wheat

Treatment	Mean root length per seed (mm.)
Control	61·8
5% gamma-BHC	42·4
10% gamma-BHC	25·5
20% gamma-BHC	18·6
S.E. (single replicate)	6·6
Coeff. of variation	17·9%
Sig. diff. between treatments ($P = 0{\cdot}05$)	8·2

The differences in root length between treatments were found to be highly significant, while the cytological results obtained were very similar to those of expt. II. Treatment with 5 per cent. gamma-BHC dust resulted in a much reduced rate of division and the appearance of a few abnormal mitoses. There were, however, many normal mitoses at this rate of treatment.

As in rye, 10 per cent. and 20 per cent. dusts caused polyploidy, the frequency and degree of which increased with gamma-BHC concentration and which was accompanied by enlarged interphase nuclei of highly irregular outline, containing increased amounts of nucleolar material. There were few normal mitoses at the 10 per cent. rate and none at 20 per cent. As before, prophases became less frequent at the higher rates of treatment, and anaphase was totally suppressed at the 20 per cent. level.

DISCUSSION

It is clear that under the above experimental conditions gamma-BHC exerted a marked effect on the growth of wheat and rye, while cress appeared to be unaffected.

This differential effect has, however, been established only for the conditions of these experiments, namely, the application of a standard weight of treatment dust to a standard volume of seed.

The stimulation of growth with 0·1 per cent. gamma-BHC content dust observed in the first experiment has been found on occasion in other experiments, but we have been unable either to obtain this effect consistently or to define the requisite conditions and it must therefore, for the present, be considered fortuitous.

In expts. I and II significant reduction of growth and mitotic aberrations were induced in wheat and rye respectively, by approximately the same concentrations of gamma-BHC. In expt. III on wheat, the threshold concentrations of gamma-BHC for inhibition of root growth and for the production of abnormal cytological effects appeared to be the same.

It is, therefore, reasonable to conclude that the growth-inhibiting and mitotic actions of gamma-BHC are causally related.

The cytological effects of the compound resemble closely those of colchicine (Levan, 1938), acenaphthene (Kostoff, 1938), isopropylphenylcarbamate (Doxey, 1949), and other compounds inducing spindle inhibition, and, as such, are distinct from the radiomimetic effects of mustard gas (Koller, 1947), the nitrogen-mustards (Novick and Sparrow, 1949), 2-methyl-4-chlorophenoxyacetic acid (Doxey and Rhodes, 1949), &c. In the latter group, many of the effects which are manifested during mitosis (i.e. at metaphase and anaphase) can be shown to have originated during the preceding interphase (Koller, 1947).

The absence of chromosome breaks in our experiments and their rarity in those of Kostoff suggest that gamma-BHC is unlikely to cause any considerable genetical effects.

It should be emphasized that the effects described above occur in seedlings grown on agar or on moist filter-card. The action of the compound (on growth at least) appears to be very much less marked on plants growing in soil, and there is no evidence that it brings about a permanent reduction of

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growth in the field when concentrations and rates of application normally recommended for insecticidal purposes are used.

LITERATURE CITED

DOXEY, D., 1949: The Effect of Isopropyl Phenyl Carbamate on Mitosis in Rye (*Secale cereale*) and Onion (*Allium cepa*). Ann. Bot., N.S. xiii. 329–35.

—— and RHODES, A., 1949: The Effect of the Plant Growth Regulator, 4-chloro-2-methylphenoxyacetic Acid on Mitosis in the Onion (*Allium cepa*). Ibid. xiii. 105–11.

JOHN INNES HORTICULTURAL INSTITUTION: Thirty-sixth Annual Report (for the year 1945), x.

KOLLER, P. C., 1947: The Experimental Modification of the Nucleic Acid Systems in the Cell. *Symposia of the Society for Experimental Biology*, i. 270–90.

KOSTOFF, D., 1938: Irregular Mitosis and Meiosis Induced by Acenaphthene. *Nature*, cxli. 1144–5.

—— 1948: Atypical Growth, Abnormal Mitosis, Polyploidy and Chromosome Fragmentation Induced by Hexachlorocyclohexane. Ibid. clxii. 845–6.

LEVAN, A., 1938: The Effect of Colchicine on Root Mitosis in the Onion. *Hereditas*, xxiv. 471–86.

NOVICK, A., and SPARROW, A. M., 1949: The Effect of Nitrogen Mustard on Mitosis in Onion Root Tips. *J. Heredity*, xl. 13–17.

QUIDET, P., and HITIER, M., 1948: (Production of Polyploid Plants by Treatment with Hexachlorocyclohexane and Polychlorocyclane sulphide). (In French.) *C.R. Acad. Sci., Paris*, ccxxvi. 833–5.

Development of Perithecia and Reproductive Structures in Two Species of *Ceratocystis*

BY

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With Plate VI and four Figures in the Text

ABSTRACT

Two species of the fungus *Ceratocystis* are described which have been isolated from beetle galleries in Japanese larch. The detailed development of reproductive structures is described in each, confirming the work of Varitchak in the case of *C. piceae*. *C. autographa* is diagnosed as a new heterothallic species.

ANDRUS and Harter (1933) expressed doubt at Varitchak's observations (1931) on the presence of ascogenous hyphae and their subsequent development in *Ceratostomella piceae* Münch, but Gwynne-Vaughan and Broadhead (1936) showed that the development of ascogenous hyphae in *C. fimbriata* was similar to that described by him. The present study is essentially to confirm the work of Varitchak (1931) on the reproductive structures in *Ceratocystis*[1] *piceae* (Münch) Bakshi comb. nov. (= *Ceratostomella piceae* Münch). Development of reproductive structures and the life-history have also been studied in a new species of *Ceratocystis*, *C. autographa*.

C. piceae was isolated from the gallery of the ambrosia beetle, *Trypodendron lineatum*, infesting Japanese larch. The standing trees were dying over a wide area in a forest at Blair Atholl, Perthshire, Scotland, during 1946–7. The trees were also infested with bark-beetles, *Dryocoetes autographus* and *Hylurgops palliatus*, and two new species of *Ceratocystis*, *C. autographa* and *C. galeiformis*, were isolated from their galleries.

Mycelia, conidia, and perithecia of *C. piceae* and *C. autographa* developed in the Trypodendron gallery and under the bark respectively. Cultures were started by transferring fungus hyphae, conidia, or ascospores from the perithecial base into agar. The technique described by Gregor (1932) was successfully employed in making monoascospore cultures of the fungi. Cultural characters were studied on 2 per cent. malt agar in tubes and Petri dishes kept in darkness at 22° C. For studying the development of perithecia and asci, the fungi were grown on malt agar in Petri dishes, and when all stages from immature to mature perithecia developed the agar block was cut into small discs and these were fixed for twenty-four hours in Flemming's weak solution.

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[1] The nomenclature of *Ceratostomella* Sacc., *Ceratocystis* Ell. and Halst., and other allied genera is given by the author elsewhere (Bakshi, 1950).

[Annals of Botany, N.S. Vol. XV, No. 57, January, 1951.]

Sections were cut in paraffin, 6–10 μ thick, bleached in hydrogen peroxide, and stained either in iron-alum-haematoxylin with erythrosin in clove oil as a counter-stain or in Flemming's triple stain. Observations were also made from mycelium producing perithecial rudiments and squashed preparations of perithecia, stained in lacto-phenol cotton blue.

Ceratocystis piceae (Münch) Bakshi comb. nov.

This species is homothallic and produces conidia, coremia, and perithecia in single ascospore culture.

Ascogonia and antheridia have not been observed in any of the numerous cultures examined. The primordia of ascocarps were first recognized by a coil of hyphae within the cavity in which ascogenous hyphae developed.

The perithecial wall consists of two layers (Pl. I, 2): an outer one consisting of thick-walled, dark brown to black hyphae, 3–4 layers thick, and an inner one of large thin-walled cells, 4–5 layers in thickness. The base of the perithecium is occupied by 5–7 rows of small, thin-walled 'cushion cells' containing a nucleus in each. These two layers correspond to the two outer layers in the perithecial wall in *Ceratostomella fimbriata* described by Gwynne-Vaughan and Broadhead (1936), but in *C. fimbriata* the latter workers describe the cushion cells as forming one or two 'layers' lining the perithecial cavity.

While the perithecium is still young, the neck emerges as a cone of thin-walled parallel hyphae and the latter converge at the apex leaving an ostiole, about 7–8 μ in diameter (Pl. I, 2). The two layers of the perithecial base extend into the neck to form an outer layer of thick-walled hyphae, 4–5 cells thick, and an inner layer of thin-walled cells (Pl. I, 1, 2). The latter disintegrate when the perithecium matures, leaving a central cavity 5–6 μ broad (Pl. I, 1). The outer thick-walled, dark brown cells of the neck become thin-walled and hyaline towards the top. When mature, a few of these open out to form a crown of cilia, 16–22 in number.

The ascogenous hyphae arise from the cushion cells situated only at the base of the perithecium (Pl. I, 3). In *C. fimbriata* Gwynne-Vaughan and Broadhead have shown that the ascogenous hyphae grow inwards from every direction since the cushion cells line the perithecial cavity. The apices of the ascogenous hyphae lie free in the perithecium and never attach themselves to the wall, as was also observed by Varitchak (1931) in *C. piceae*. The ascogenous hyphae are branched and at first very narrow but later up to 2 μ broad. When young, they are coenocytic and the nuclei lie without any association (Text-fig. 3, *a*, *b*). In later stages (Text-fig. 3, *c*, *d*) the nuclei become associated in pairs and each pair is separated from the other by a transverse wall. Varitchak (1931) did not consider them as dicaryons because they were exceptional and rare and regarded the approach of the two nuclei as accidental. In the present study, paired nuclei were common in all the preparations during later stages in the development of the ascogenous hyphae. The terminal portion of the ascogenous hypha which contains two nuclei then bends over forming the crozier (Text-fig. 3, *e*) and one nucleus remains in the straight portion of the hypha and the other passes to the tip (Text-fig. 3, *f*). The two nuclei then undergo conjugate division resulting in four nuclei (Text-fig. 3, *g*, *h*). Walls are laid down so as to make the terminal and the basal cells each uninucleate and the penultimate cell binucleate (Text-fig. 3, *i*). The two nuclei in the penultimate cell then approach towards each other, become surrounded by a common vesicle (Text-fig. 3, *j*), and ultimately fuse (Text-fig. 3, *k*) to form the fusion nucleus of the ascus (Text-fig. 3, *l*). Whether the ultimate and the antepenultimate cells fuse to form a second ascus as has been suggested by Varitchak (1931) was not observed. The fusion-nucleus is distinguished from the nucleus in the ascogenous hypha by the larger size of the former. The portion of the hypha containing the fusion-nucleus broadens to 6–7 μ.

The fusion-nucleus forms a chromatin network within the nuclear vesicle (Text-fig. 3, *m*). Though the immediately succeeding stages were rare, it is evident from Text-fig. 3, *n*, *o*, that the nuclear reticulum breaks up into four chromosomes, of which two pass to one pole and two to the other. Thus the first division of the fusion nucleus in the ascus is a reduction division and the fusion nucleus is interpreted as diploid, other sexual fusions being absent, as demonstrated by Varitchak (1931). The binucleate stage of the ascus is followed by four- and eight-nucleate stages (Text-fig. 3, *q*, *r*, *s*). The ascus has a delicate wall and may be round or oval, 6–7·5 × 5–6 μ. From numerous sections it seems that the asci are cut off from the ascogenous hyphae soon after they are formed. The asci lie free in the perithecial cavity for some time till their walls dissolve away and the ascospores are set free. Sections through mature perithecia reveal the presence of ascospores and indicate that after the asci are formed the bases of the ascogenous hyphae dissolve away forming the nutritive matrix. Ascospores are crescent-shaped (Text-fig. 3, *t*), hyaline, 1-celled, 4–5 × 1–2 μ, average 4·5 × 1·4 μ. Paraphyses and periphyses do not develop in the perithecium.

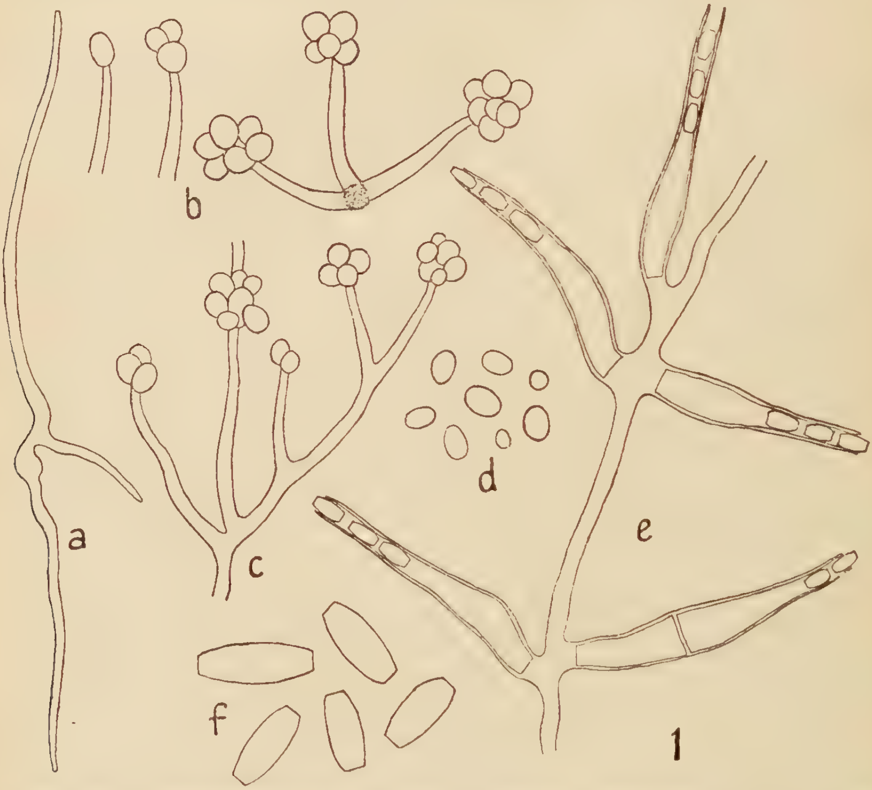
Ceratocystis autographa Bakshi sp. nov.

Cultural studies. On agar, *C. autographa* is slow growing and the rate of radial growth is about 7–8 mm. in 10 days. The mat is mostly appressed to the substratum except at the inoculum where the aerial mycelium is usually well developed (Pl. I, 6). The mycelium is colourless when young, but with age the general mat becomes blackish-grey.

Conidia are borne on aerial hyphae both exogenously and endogenously. In the former case the conidia are borne singly or in groups of 3–4 or more at the apex of short hyphae arising from the substratum (Text-fig. 1, *b*). In later stages they are also borne on aerial strands of hyphae either directly or on short branches given off from them (Text-fig. 1, *c*). Conidia are round or oval, 1-celled (Text-fig. 1, *d*), hyaline, 2·7–6·6 × 1·6–2·3 μ, average 3·7 × 2·1 μ.

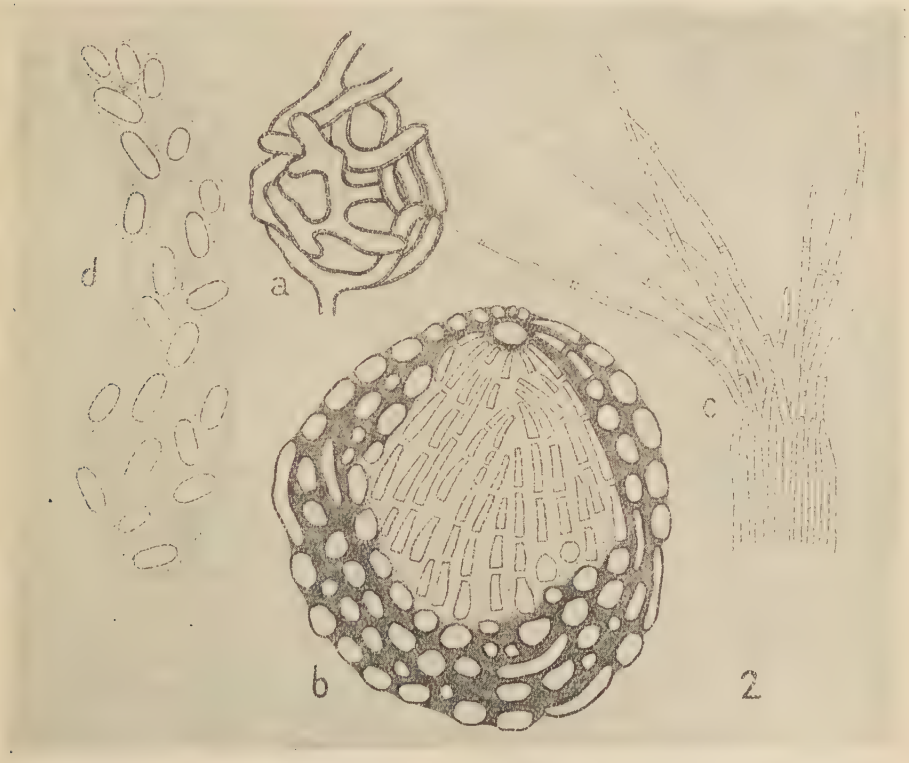
Endoconidia are borne within conidiophores, which develop laterally from the main hypha (Text-fig. 1, *e*). Conidiophores are up to 30 μ long, 3·5 μ broad at the base and 1·7 μ at the top. They are thick-walled, brown, usually unbranched, and cut off from the main hypha by a transverse septum at the base, though a second septum may occasionally appear in the upper portion. Conidia are formed within them in chains. They are barrel-shaped, thin-walled, 1-celled (Text-fig. 1, *f*), hyaline, 3·5–6·8 × 1–2 μ, average 4·9 × 1·5 μ.

Perithecia develop abundantly in polysporous cultures (Pl. I, 6). A young perithecium is dark brown, but becomes black on maturity. The venter of the perithecium is more or less globular. The ventral diameter and height vary from 88–182 μ and 78–165 μ respectively, averages being 144 μ and 137 μ. The base is hirsute. The neck measures 333–504 μ, average 422 μ. The breadth of the neck at the base varies from 22–32 μ, average 28 μ, that at the top 10–17 μ, average 14 μ.



TEXT-FIG. 1. *Ceratocystis autographa* Bakshi. *a*, germinating ascospores (× 570); *b*, conidia borne exogenously singly or in heads on short hyphae arising from substratum (× 1,400); *c*, same, showing conidia borne on aerial hyphae (× 1,400); *d*, conidia borne exogenously (× 1,400); *e*, aerial hypha showing endoconidiophores bearing endoconidia in chains (× 1,750); *f*, endoconidia (× 3,100).

When the perithecium is mature, the hyaline tip cells of the neck open out to form the crown of cilia (Text-fig. 2, *c*). Their number varies from 8 to 32. They are from 10 to 70 μ long, average 35 μ, 1·7 μ broad at the base, and 1·2 μ at the tip. The cilia are thin-walled, hyaline, and many-celled. The ascospores collect at the ostiole in the form of a globule, milk-white in colour at first but turning yellow with age. The asci are round or elongate (Text-fig. 4, *n*), possess the usual delicate wall, and measure 9 × 3·5 μ. The ascospores are hyaline, 1-celled, elongate (Text-fig. 2, *d*) and measure 3–4 × 1–1·5 μ, average 3·5 × 1·3 μ. Ascospores germinate by two or more germ-tubes (Text-fig. 1, *a*.)

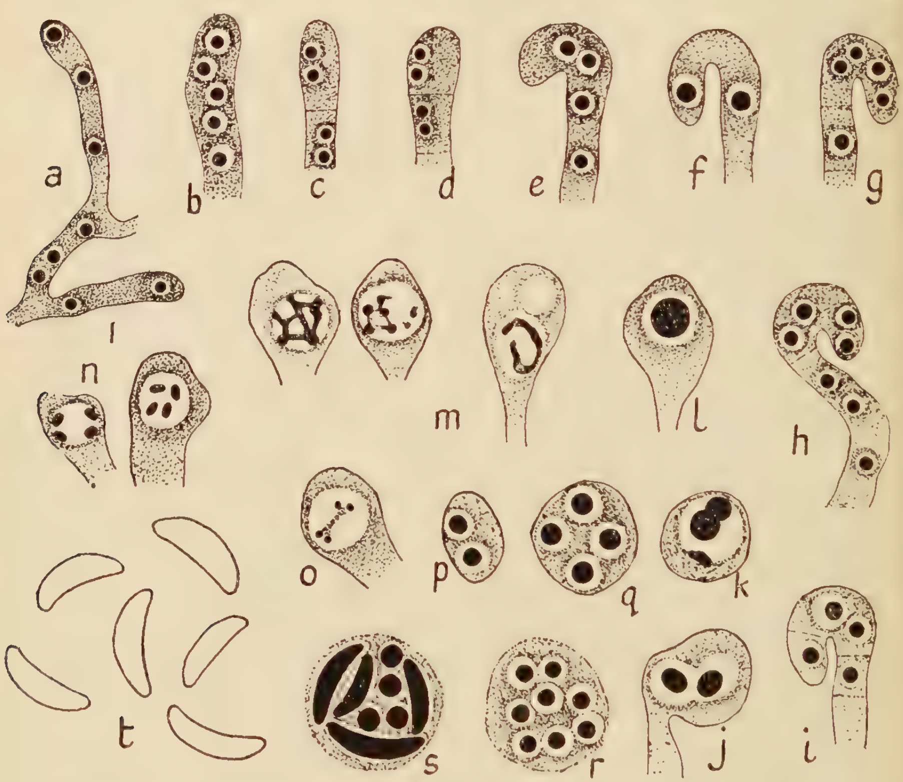


TEXT-FIG. 2. *Ceratocystis autographa* Bakshi. *a*, coiling of hyphae before initiation of perithecium (× 1,750); *b*, longitudinal section of young perithecium cut obliquely showing two sheaths of perithecial wall and ostiole (× 960); *c*, top of the neck showing crown of cilia (× 960); *d*, spores-tendril with ascospores embedded in mucilage (× 3,100).

Sclerotia develop abundantly. They are dark brown to black bodies, round, and 60 μ in diameter. Young hyphae are thin-walled, hyaline, and 1–2·5 μ broad. They become thick-walled and brown with age and may be up to 4 μ broad.

The fungus agrees closely with *Ceratostomella paradoxa* Dade (Dade, 1928), but differs from the latter in cultural characters as well as conidial, perithecial, and spore measurements and in the absence of appendages on the perithecial base in the present species.

Technical description. Mycelium slow-growing on culture media, appressed with aerial mycelium at the inoculum; young hyphae thin-walled, hyaline, 1–2·5 μ broad, old hyphae thick-walled, brown, up to 4 μ broad; conidia exogenous or endogenous: former on hyphae in heads, conidia round or oval, 1-celled, hyaline, 2·7–6·6 × 1·6–2·3 μ, average 3·7 × 2·1 μ, endoconidia within conidiophores which are narrowly flask-shaped, up to 30 μ long, 3·5 μ broad at base, 1·7 μ at top, thick-walled, brown, septate, endoconidia in chains, barrel-shaped, thin-walled, 1-celled, hyaline, 3·5–6·8 × 1–2 μ, average 4·9 × 1·5 μ; perithecia black, globose, hirsute, diameter of base 88–182 μ, average, 144 μ, height of base 78–165 μ, average 137 μ; length of neck 333–504 μ, average 422 μ, diameter of neck at base 28 μ, at top 14 μ; ostiole with 8–32 cilia, cilia septate, hyaline, thin-walled, 10–70 μ, 6–8 celled, average 35 μ long, 1·7 μ broad at base, 1·2 μ at tip; asci deliquescent, round or elongate, 9 × 3·5 μ; ascospores 8, hyaline, 1-celled, elongate, 3–4 × 1–1·5 μ, average 3·5 × 1·3 μ; sclerotia dark brown to black, round, 60 μ in diameter.



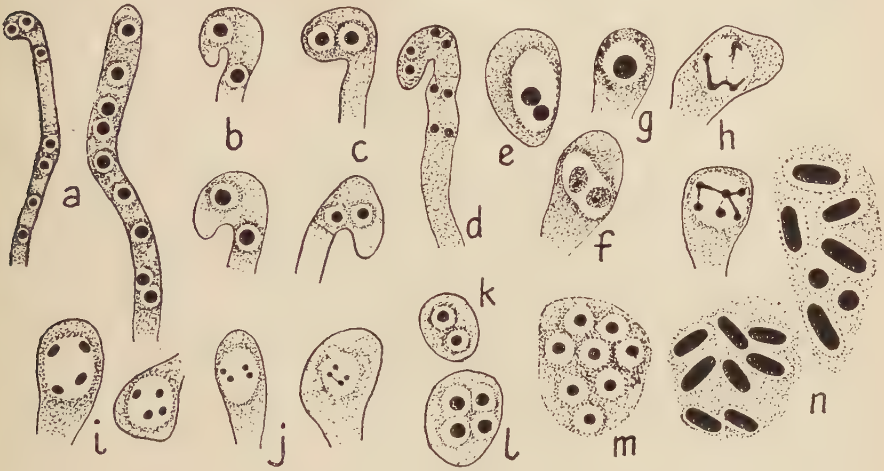
TEXT-FIG. 3. *Ceratocystis piceae*: development of ascogenous hyphae and asci.

Fungus heterothallic. In *Larix leptolepis*, under the bark, associated with *Dryocoetes autographus* and *Hylurgops palliatus*, Blair Atholl, Perthshire, Scotland.

Latin Diagnosis: Myceliis tarde crescentibus, substrato appressis; conidiis exogenis vel endogenis; exoconidiis ad formam in genere *Cephalosporio*, hyalinis, globosis vel ovoideis, unicellularibus 2·7–6·6 × 1·6–2·3 μ; endoconidiophoris ampulliformibus, brunnescentibus, crasso-tunicatis, 30 μ longis, basi 3·5 μ latis, apice 1·7 μ latis; endoconidiis catenulatis, cylindricis vel doleiformibus, tenui-tunicatis, hyalinis, unicellularibus 3·5–6·8 × 1–2 μ; peritheciis nigris, globosis, hirsutis; perithecii ventre 88–182 μ lato, 78–165 μ alto; rostello 333–504 μ longo, 14–28 μ lato; ostioli filamentis 8–32, 6–8 cellularibus, hyalinis, 35 μ longis; 1·2–1·7 μ latis; ascis evanidis, globosis vel elongatis, 9 × 3·5 μ; ascosporis hyalinis, cylindricis, unicellularibus, 3–4 × 1–1·5 μ; sclerotiis ± globosis, 60 μ latis.

Fungus heterothallicus, in truncis *Laricis leptolepidis.*

Heterothallism. The fungus is heterothallic and produces only conidia and sclerotia in monoascospore cultures. Perithecia are formed when two mycelia of opposite strains are mated (Pl. 1, 7).



Text-fig. 4. *Ceratocystis autographa*: development of ascogenous hyphae and asci.

Development of the perithecium and the neck. The development follows the same course as described for *C. piceae*. The primordium of the perithecium is recognized by a coil of hyphae (Text-fig. 2, *a*). The perithecial wall is distinguished into two layers. Cushion cells occupy the base of the perithecium (Pl. I, 5). The initial stage of the neck is laid down very early in the development of the perithecium. Text-fig. 2, *b* shows the rudiment of a perithecium where the hyphae are seen to converge at the neck leaving a small ostiole. A transverse section of the neck (Pl. I, 4) shows a central canal, 5 μ broad on the average, surrounded by five layers of thick-walled cells.

Development of ascogenous hyphae and asci. The ascogenous hyphae arise from the cushion cells only (Pl. I, 5). They are at first very narrow and have no cross walls. The nuclei lie free in the cytoplasm without any association (Text-fig. 4, *a*).

As the perithecium develops, the ascogenous hyphae become broader (up to 2 μ broad). Their apices are never attached to the perithecial wall. When the ascogenous hyphae have fully developed, the tips bend over to form croziers (Text-fig. 4, *b*, *c*, *d*). Text-fig. 4, *d* shows the nuclei lying in pairs side by side along the breadth of the hypha. The hypha at the region of the crozier has always been found to be broader than that at other regions. It appears, therefore, that the crozier formation is a device for allowing the two nuclei at the terminal portion of the ascogenous hypha to come to lie side by side. Hence when the pair of nuclei divide by conjugate division, the pre-fusion nuclei, from opposite strains, would be included in the sub-terminal cell. This stage was not observed, but from the study of *C. piceae* (Text-fig. 3, *i*) it is assumed that similar development takes place in *C. autographa*. Fusion between the two nuclei in the ascus is preceded by their inclusion within a common vesicle (Text-fig. 4, *e*, *f*). From the figure it can be seen that a nucleolus is contained within each of the prefusion nuclei.

The fusion nucleus (Text-fig. 4, *g*), on division, forms the nuclear reticulum (Text-fig. 4, *h*) within the vesicle. The reticulum finally breaks up into four chromosomes, of which two pass to one pole and two to the other (Text-fig. 4, *i*, *j*). The two-nucleate stage in the ascus is followed by four- and eight-nucleate stages (Text-fig. 4, *l*, *m*). The immature ascus possesses the usual delicate wall. The nuclei are at first round but elongate on maturity, and the ascospores are formed around them (Text-fig. 4, *n*).

The delicate walls of the asci soon dissolve away so that the ascospores lie free in the perithecial cavity embedded in mucilage. When a mature perithecium is placed in water on a slide and observed under the microscope, the ascospores are liberated in the form of tendril (Text-fig. 2, *d*). The latter keeps its form since the mucilage, in which the spores are embedded, is immiscible in water.

SUMMARY

Ceratocystis piceae (Münch) Bakshi is self-compatible, bearing conidia, coremia (graphia), and ascospores in perithecia in single ascospore cultures.

No sexual organs have been observed, and the initial of the perithecium is first recognized by a coil of hyphae.

The development of the perithecium and the neck has been described.

The ascus develops after the fusion of two nuclei in the penultimate cell of the ascogenous hypha.

Four chromosomes are seen in the first division of the fusion nucleus in the ascus, two of which later pass to one pole and two to the other. This division being meiotic, the fusion nucleus of the ascus is interpreted as diploid with four chromosomes, assuming that no fusion occurs previous to the one in the ascus. The haploid number is therefore two. The ascus is deliquescent.

The life-history of *Ceratocystis autographa* Bakshi, isolated from the bark of Japanese larch and associated with the bark-beetles *Dryocoetes autographus* and *Hylurgops palliatus*, is described. Asexual reproduction is accomplished by two conidial forms, borne exogenously and endogenously.

C. autographa is heterothallic, producing conidia and sclerotia in monoascospore cultures. Perithecia are formed on mating two mycelia of opposite

strains. Development of the perithecium and reproductive structures follows the same course as described for *C. piceae.*

The author wishes to express his indebtedness to Dr. Malcolm Wilson under whose guidance the work was carried out in Edinburgh. His sincere thanks are due to Professor Sir W. Wright Smith for the Latin diagnosis of the new species and to Mr. E. W. Mason for the advice on the nomenclature of *Ceratocystis.* Cultures of the new species and of *C. piceae* have been deposited at the Commonwealth Mycological Institute, Kew, Forest Products Research Laboratory, Princes Risborough, and Botany Department, Edinburgh University.

LITERATURE CITED

ANDRUS, C. F., and HARTER, L. L., 1933: Morphology of Reproduction in *Ceratostomella fimbriata.* J. agric. Res., xlvi. 1059–78.

BAKSHI, B. K., 1950: Studies on four species of *Ceratocystis* with a Discussion on Fungi causing Sap-stain in Britain. Mycol. Paper, Commonw. Mycol. Instit. (in press).

DADE, H. A., 1928: *Ceratostomella paradoxa*, the Perfect Stage of *Thielaviopsis paradoxa* (de Seynes) von Höhnel. Trans. Brit. mycol. Soc., xiii. 184–94.

GREGOR, Mary, J. F., 1932: A Study of Heterothallism in *Ceratostomella pluriannulata* Hedgcock. Ann. mycol., Berl., xxx. 1–9.

GWYNNE-VAUGHAN, H. C. I., and BROADHEAD, Q. E., 1936: Contribution to the Study of *Ceratostomella fimbriata.* Ann. Bot., l. 747–58.

VARITCHAK, B., 1931: Contributions à l'étude du développement des Ascomycètes. Botaniste, xxiii. 1–184.

DESCRIPTION OF PLATE VI

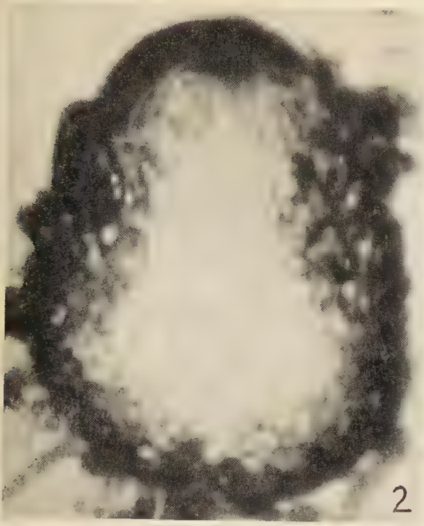
Illustrating the paper on *Ceratocystis* by B. K. Bakshi.

Ceratocystis piceae (Münch) Bakshi

1. Transverse section of neck of perithecium showing outer thick-walled cells and central canal. (× 1,000.)
2. Longitudinal section of young perithecium showing outer sheath of thick-walled hyphae converging at the top leaving a small ostiole. Inner sheath of thin-walled hyphae extend above as thin-walled cells of the neck. (× 650.)
3. Longitudinal section of mature perithecium showing perithecial wall distinguished into two layers. Cushion cells occupy base of perithecium, from which ascogenous hyphae arise. (× 500.)

Ceratocystis autographa Bakshi

4. Transverse section of the neck of the perithecium showing outer thick-walled cells. (× 1,000.)
5. Longitudinal section of mature perithecium showing two sheaths of perithecial wall, cushion cells at the base of perithecium and ascogenous hyphae. (× 620.)
6. 45-day-old culture on malt agar. Note the abundant perithecia. (× 1½.)
7. Pairing of two monoascospores cultures from opposite strains showing perithecia at line of union. (× 1½.)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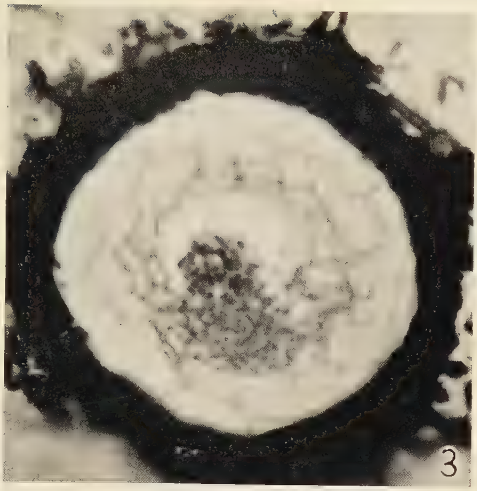
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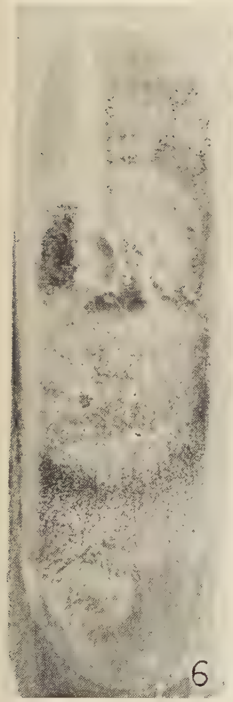
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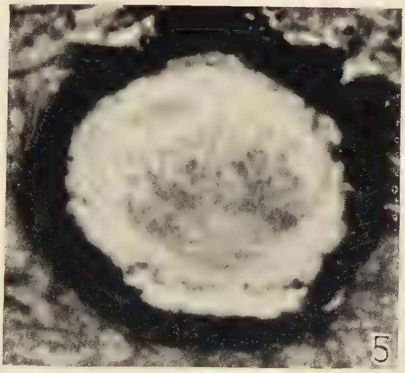
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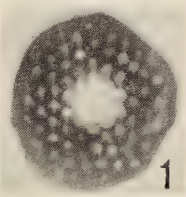
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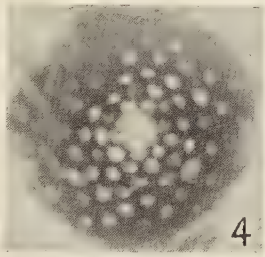
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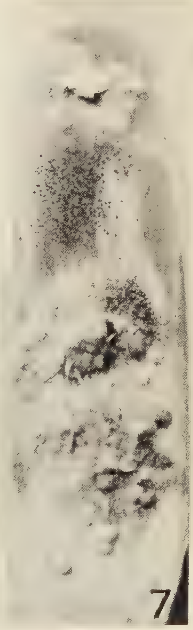
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B. K. BAKSHI

Physiological and Ecological Studies in the Analysis of Plant Environment

VI. The Constancy for Different Species of a Logarithmic Relationship between Net Assimilation Rate and Light Intensity and its Ecological Significance

BY

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AND

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With twenty-two Figures in the Text

ABSTRACT

In this further study of light as an environmental factor the effects of shading on the growth of *Helianthus annuus*, *Fagopyrum esculentum*, *Trifolium subterraneum*, *Tropaeolum majus*, *Lycopersicum esculentum*, *Vicia faba*, *Pisum sativum*, *Hordeum vulgare*, *Solanum dulcamara*, and *Geum urbanum* have been investigated.

It has been established that the reductions in the net assimilation caused by shading are similar for all ten species. The net assimilation rate during the season of active growth is linearly related to the logarithm of the light intensity. When similar experiments are conducted late in the autumn and the relative growth rate is very low, the logarithmic relationship no longer holds.

From the data it is possible to obtain precise estimates of the compensation point. The mean values of the compensation point ranged from 0·06 to 0·09 of daylight for eight species, while for *V. faba* and *H. vulgare* somewhat higher figures were obtained—0·14 and 0·18 of daylight.

Fluctuations in the net assimilation rate in full daylight showed no correlation with variations in the value of the compensation point.

From these results it is concluded that species cannot be grouped into 'sun' or 'shade' plants, either on the basis of differences in the value of the compensation point or on the grounds that there are large variations in the effects of shading on net assimilation rate.

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INTRODUCTION

IN previous studies relating to the autecology of *Scilla non-scripta* the effects of shading on seasonal growth and development were investigated by the techniques of growth analysis. It was established that over the range of normal daylight to 0·1 daylight there were linear relationships between the logarithm of light intensity and (i) the ratio of leaf area to leaf weight, (ii) the ratio of total leaf area to total plant weight, and (iii) the net assimilation rate (Blackman and Rutter, 1948, 1950). In addition, it was demonstrated that the relative growth rate (efficiency index) bore a curvilinear—but not logarithmic—relation to light intensity.

In view of the ecological and physiological importance of these findings for the interpretation of the effects of light intensity on growth, it seemed desirable that the investigations should be extended to determine whether these relationships also held for other species. In the first place the bluebell is a somewhat specialized plant, since neither the total leaf weight nor the inflorescence weight is changed by shading. Secondly, the ratio of leaf area to total plant weight is small and growth is very slow. Thirdly, the experiments only covered the phase of active growth of this species, namely, the months of April and May and, in consequence, no information was available as to how far such relationships are limited to the light conditions ruling during spring.

This and subsequent papers will be concerned with a further analysis of the effects of shading on the growth of some nine other species. The present paper is primarily a study of the logarithmic relationship between light intensity and net assimilation rate.

The investigation falls roughly into two parts: field experiments carried out by the first author during 1940–3 at Slough, Bucks., and pot experiments at Oxford over the period 1947–8. The Slough experiments aimed at studying the effects of shading on the growth of *Helianthus annuus* at different periods of the year. The work at Oxford, besides extending and amplifying the observations on the sunflower, was expanded to cover both other species which normally grow in full daylight (*Hordeum vulgare*, *Vicia faba*, *Pisum sativum*, *Fagopyrum esculentum*, *Tropaeolum majus*, *Trifolium subterraneum*) and species which are associated with shady habitats (*Geum urbanum*, *Solanum dulcamara*).

Experimental Methods

(*a*) *Design of experiments.*

Field experiments. All the Slough experiments were carried out on a single experimental site in the middle of an open field where the soil consisted of Thames gravel. There were four levels of light intensity: one series of plots received full daylight and the others were shaded by means of light wooden frames covered either with butter muslin or sheets of zinc, perforated with a varying number and size of hole. Each frame was supported some 12 in. off the ground, and in order to prevent rain dissolving traces of zinc, the perforated sheets were painted with thick vaseline dissolved in petrol.

The size of each frame had perforce to be kept small, since otherwise they would have been very unwieldy to lift off and on. Moreover, a small size had the advantage that possible indirect effects of shading, especially the raising in the air temperature, would be minimized by diffusion and convection currents. The replication of the screens was fivefold in a random block arrangement.

Each of the light treatment plots was split into half, and one half prior to sowing received a mixture of ammonium sulphate, superphosphate, and potassium sulphate equivalent respectively to 2, 4, and 2 cwt. per acre. Sunflower seed was sown in rows a foot apart, and as soon as the seedlings appeared above ground the plants were singled to 6 in. apart in the row. For the field experiments a 'semi-dwarf' variety 'Jupiter' was employed, but in the subsequent pot experiments another similar variety 'Pole Star' was substituted. The suitability of these varieties for the conditions of Great Britain has been discussed elsewhere (Blackman, 1946).

In order to obtain comparable results between experiments, the plots were first sampled and the screens put in place when the plants had reached a standard size, i.e. the second pair of true leaves had started to expand. The second sample was normally taken when it was judged that the plants growing in full daylight had at least doubled their initial weight.

When numbers permitted eight plants were selected at random from each plot at each sampling occasion. The individual plants were carefully dug up with a trowel and transported, with the ball of soil attached, to the laboratory, where the soil was washed from the roots. The plants were then divided with scissors into leaves with the petioles attached, shoot, and root (i.e. any portion of the plant below soil-level). A sub-sample of leaves was taken for the estimation of leaf area and the individual parts dried at 100° C. and weighed.

Pot experiments. The procedure of the field experiments was adopted in the pot experiments. Standard glazed earthenware pots, of 10-in. diameter with a basal drain hole at the side, were employed in all experiments. The pots were filled with a mixture of soil and sand, and so as to ensure that growth should not be limited by a deficiency of major nutrient elements, ammonium nitrate, ammonium phosphate, and potassium sulphate were applied in solution to the soil surface. The pots then received more water, and the seed was sown. After emergence the plants were reduced to a standard number per pot, the actual number depending on the species—normally 5–6 plants.

All the pots received full daylight until the plants had reached 'standard' size, then they were matched by eye into groups of two or more pots (the number depending on frequency of sampling) and each group assigned at random to a light treatment. At the time when the screens were placed in position in a random block arrangement the appropriate number of pots was withdrawn for the determination of leaf area and the individual dry weights of the root, stem, and leaves. Depending upon the design of the experiment, the replication of pots for each light treatment was four- to sevenfold.

For the separation of the roots from the soil a procedure somewhat different from that of the field experiments was adopted, since it was considered that in case of species with a less 'fibrous' root system than the sunflower the recovery would be less complete. After removing the plants and the main roots from the pot, the soil-sand mixture was washed through a sieve which retained the roots. In this way the loss of roots was slight, consisting only of the finer extremities. A small amount of soil, mainly sand particles, adhered to the roots, and in one sample of buckwheat where it was expected that the retention was unusually high, actual estimation showed that the contamination was only 3·8 per cent. by weight. It is therefore concluded that the errors of estimating root weight were small and that loss of the finer roots and contamination with sand of the remainder were largely compensatory.

For the majority of experiments besides full daylight, there were three levels of shading, i.e. butter muslin and zinc sheets with two types of perforation. In a few experiments a screen with a higher light transmission than butter muslin was added; this consisted of galvanized iron wire mesh.

(*b*) *Estimation of leaf area*

Two methods of estimating leaf area have been employed in this investigation. For the first series of experiments at Slough, immediately after the separation of the leaves from the stem, sub-samples of leaves from each plot were placed between two sheets of glass, illuminated from below, and the outlines drawn on paper of uniform thickness. The sub-sample of leaves was then dried at 100° C. and weighed, while the leaves outlined on the paper were cut out with a pair of scissors and themselves weighed along with a square of paper from the same sheet, measuring 100 sq. cm. From these three weights the ratio of the area of fresh leaf per gramme dry weight was calculated and this factor was applied to the dry weight of the whole leaf sample to determine the total leaf area.

In the experiments at Oxford the outlines of the sub-sample of fresh leaves, but without the petioles, except in experiment 1, were obtained by means of a blue print and the area subsequently determined with a planimeter. As before, from the area and the dry weight of the sub-sample the factor of leaf area to dry weight was calculated and the total leaf area estimated.

(*c*) *Measurement of light intensity*

For the estimation of the degree of shading under the different screens the methods of Atkins, Poole, and Stanbury (1937) with minor modifications were adopted. In the field experiments two Weston rectifier cells covered with screens of flashed opal glass were connected by a two-way switch to an ammeter calibrated in foot-candles. One cell was placed in a horizontal position in the open and the other under the screen level with the tops of the plants; then by means of the two-way switch almost simultaneous readings were obtained from the two cells. As the cells were not completely matched and to a small extent sensitive to temperature variations, a correction factor was obtained on each occasion by placing the cells side by side and taking comparative readings.

As a further means of standardization readings were made between 11 a.m. and 1 p.m. and only on overcast days when no shadows were detectable to the eye.

For the pot culture experiments, as the Weston apparatus was no longer available, two 'Evans' selenium photo-elecric cells covered with opal glass screens were connected to an ammeter with a two-way switch. Since it was found that the calibration of the ammeter with light intensity was not linear, and because the two cells were not precisely matched, whenever light intensities determinations were made a calibration curve was first constructed. With both cells in full daylight a series of pieces of perforated metal with known values of light transmission were placed on one cell and the reading relative to the other cell recorded. Comparable sets of readings were then obtained with one cell placed under the various screens and the light transmission values determined from the calibration curve. As before, light measurements were only made on overcast days at noon.

With the butter muslin used in these experiments it was found that the degree of shading increased slightly as the cell was moved away from the screen, and it is thought that this variation may have been due to the cloth acting as a diffusing surface. The mean values given in the various experiments for the light transmission of the butter muslin are those obtained with the cell placed at the same height as the plants.

Because of the need of carrying out these experiments in close proximity to a laboratory, the only site available was not ideal, since in the early morning and again in the evening some shadows were cast by adjacent buildings. To overcome as far as possible this light gradient the screens were set out in blocks running north and south. However, when the data were statistically analysed, the block effect was seldom significant and even the significant exceptions did not indicate a consistent light trend in any direction. Moreover, the proportion of light cut out by the buildings was very small since comparative readings of the light intensity on the experimental site and an adjacent roof-top free from all shadows showed only a 5 per cent. reduction in intensity on the site.

EXPERIMENTAL RESULTS

I. *The interrelationship between light intensity, net assimilation rate, and the stage of development of* Helianthus annuus

Williams (1946) in a critical analysis of the concept of net assimilation rate emphasizes that the traditional formula of

$$\frac{W_2-W_1}{A_2-A_1} \times \frac{\log_e A_2-\log_e A_1}{t_2-t_1}$$

rests on the basic assumption that during the experimental period (t_2-t_1) leaf area (A) increases proportionately to plant weight (W). In consequence, if this relationship departs markedly from linearity, errors in the estimation of net assimilation rate may be introduced.

Again, although the determination of net assimilation rate by the earlier workers were based on leaf areas, the time and labour involved in measuring leaf areas has sometimes led to the substitution of leaf weights. More recently Williams (1939), Tiver (1942), Tiver and Williams (1943), and Petrie and Arthur (1943) have concluded that leaf protein is a more accurate measure of photosynthetically active material. Williams (1946) concludes that the criterion of leaf protein is superior to both leaf weight and leaf area since net assimilation rate based on leaf protein maintains a constant value for a longer period of the vegetative phase. However, experimental evidence suggested that during the early stages of rapid growth the net assimilation rate remains relatively constant whichever method is adopted for the estimation.

On the basis of these investigations, it is apparent that before valid conclusions can be drawn on the relationship between net assimilation rate and light intensity two conditions must be satisfied. Firstly, if precise comparisons are to be made between the results of different experiments, then it must be established that relatively small differences in the stage of development do not affect the net assimilation rate. Secondly, it is necessary to demonstrate that over the experimental periods and within the chosen stages of development the changes in leaf area under the different light-levels are linearly related to the changes in plant weight.

In order to investigate the effect of variation in age, three successive sowings of sunflower at 7- to 8-day intervals were made in pots during May. Because of the unexpectedly cold weather the plants of the first sowing appeared above ground 2–3 days before those of the second sowing and the differences in development were therefore much smaller than those between the first and third sowing. The plants were first sampled when they had emerged some 24–5, 22–3, and 14–15 days earlier. Because of the size of the experiment, sampling was spread over 2 days, and this also applied to the final sample taken 9 days later.

The results set out in Fig. 1 clearly demonstrate the logarithmic relationship between net assimilation rate and light intensity. It is also apparent that

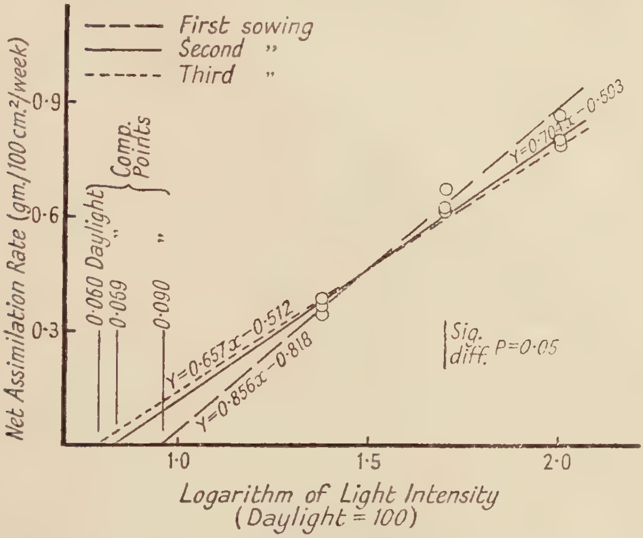


FIG. 1. Expt. 1. *Helianthus annuus.* The effects of varying light intensity on the net assimilation rate of plants of different age.

the three regression lines for the different dates of sowing are in close agreement. There is some indication that the slope of the regression line increases with the age of the plant, but the differences are not statistically significant. Thus these results are in general agreement with previous conclusions that there is little ontogenetic drift in net assimilation rate during the early period of vegetative growth.

Evidence as to the linearity of the relationship between leaf area and total plant weight is forthcoming from six sunflower experiments in which there were at least three sampling occasions. In consequence, for each light intensity it is possible to plot over consecutive periods of growth the change in total plant weight and thereby assess whether there is any major departure from a linear relationship. This procedure has been adopted in Figs. 2–7, where, in addition, the linear regressions linking net assimilation rate with the logarithm of light intensity are shown for the individual experiments.

Taking first Figs. 2–4, it is clear that the conditions for the proper estimation of net assimilation rate are satisfied since over the total experimental period the leaf area–plant weight ratios remain approximately constant for each light-level. Once more there is evidence that all the six regression lines for the two periods in the three experiments precisely link the changes in net assimilation rate with the logarithm of decreasing light intensity. In expts. 2 and 3 (Figs. 2 and 3) the assimilation rates are considerably higher in the first period than they are in the second, while in expt. 4 (Fig. 4) there is little difference.

The conditions of expts. 5 and 6 were somewhat abnormal. Owing to heavy rain the pots became waterlogged, due possibly to the silt fraction washing down to the bottom of the pots and preventing surplus water escaping through the drainage hole. In consequence, the plants made slower growth than would be anticipated for the time of the year, and in expt. 5 by the end of the fourth sampling occasion not only did the plants look unhealthy but some necrosis of the leaves had occurred. However, it is evident that over the first three sampling occasions in expt. 5 and all four occasions in expt. 6 there is an approximate linear relationship between leaf area and plant weight (see Figs. 5 and 6). Moreover, it is equally apparent in both experiments that the net assimilation rate is still proportional to the logarithm of the light intensity, even though between consecutive periods there may be appreciable changes in the actual rates, e.g. Fig. 6.

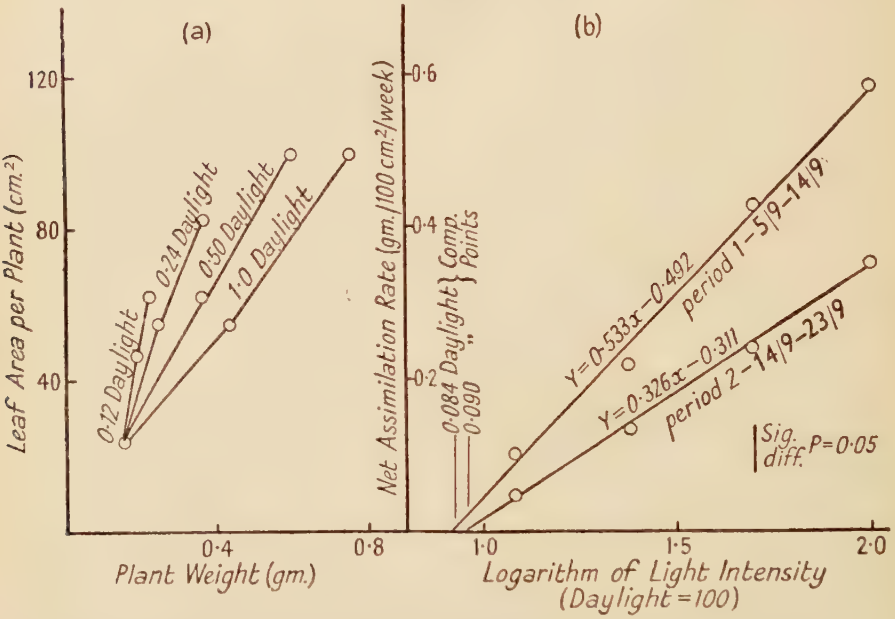


FIG. 2. Expt. 2. *Helianthus annuus.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate.

Examination of Fig. 7 (*a*) shows that the leaf area–plant weight relationship of expt. 7 is far from linear and represents, in fact, the greatest departure recorded for any of the experiments of this investigation. It is considered

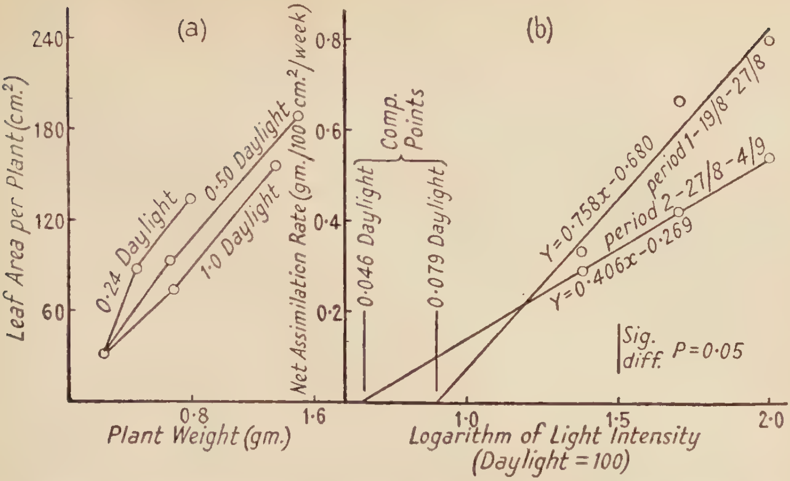


FIG. 3. Expt. 3. *Helianthus annuus.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate.

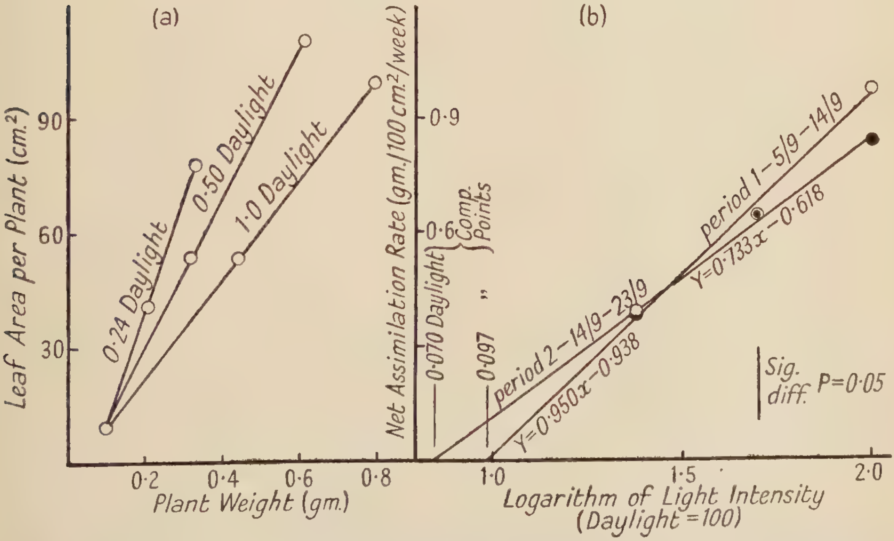


FIG. 4. Expt. 4. *Helianthus annuus.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate.

that this departure can be ascribed to the large size reached by the plants at the end of the experiment due to their very rapid growth during the experiment. With such large differences between the initial and final weights, errors are introduced in the assessment of the net assimilation rate, since there is a trend for the leaf area–plant weight ratio to diminish slowly with increasing size.

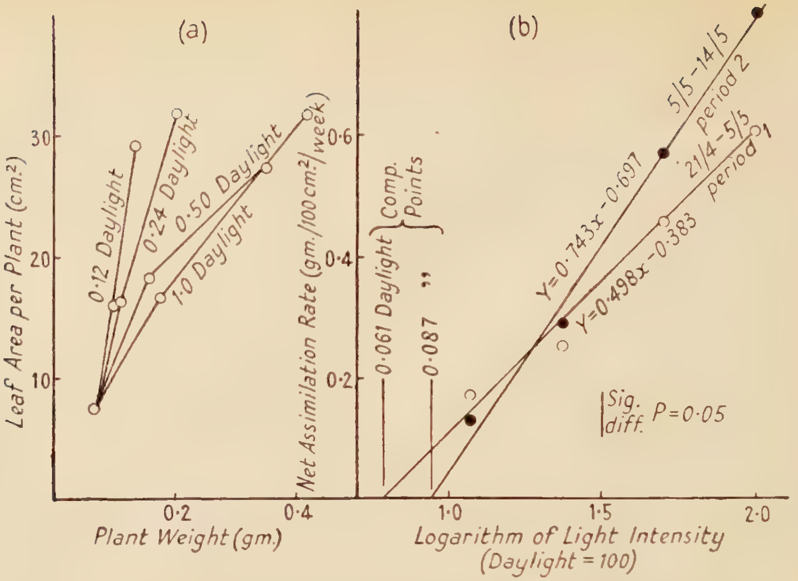


FIG. 5. Expt. 5. *Helianthus annuus*. Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate.

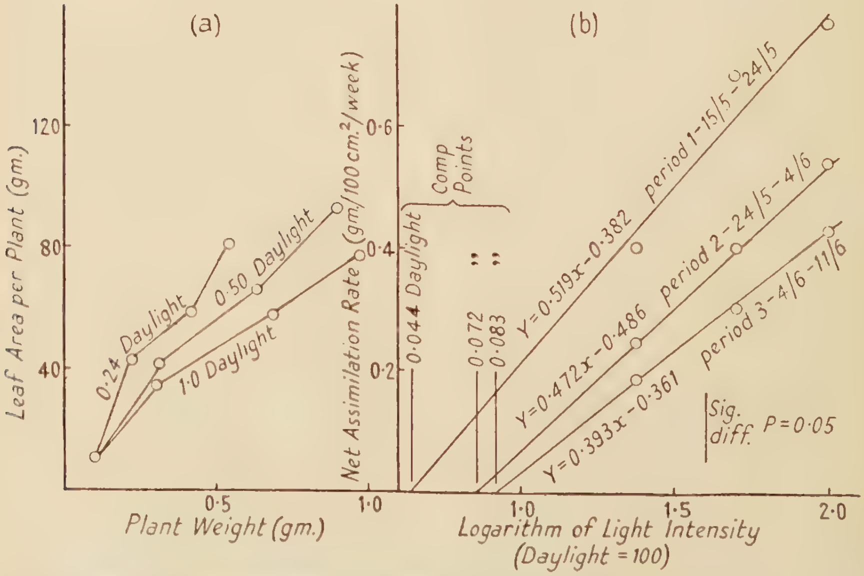


FIG. 6. Expt. 6. *Helianthus annuus*. Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate.

It is some interest to determine how far this departure from linearity affects the relationship between net assimilation rate and light intensity. The errors will be greatest if the net assimilation rates are calculated for the whole period from the data of the first and third sample and the regression of net assimilation rate against the logarithm of light intensity then computed. Conversely, the errors over the whole period will be minimized if at each light-level the net assimilation rates over the two intervals of the first and second sample and from the second to the third sample are first estimated and the regression constructed from the mean values for the two experimental periods. Fig. 7 (*c*) shows very clearly that considerable increases in the values of the net assimilation rates arise from ignoring the non-linearity of the leaf area–weight ratio over the whole period. Similarly, because of the curvilinear relationship, the net assimilation rates for the two separate periods (Fig. 7 (*b*)) must be higher than those which would be derived had there been a larger number of intermediate samples.

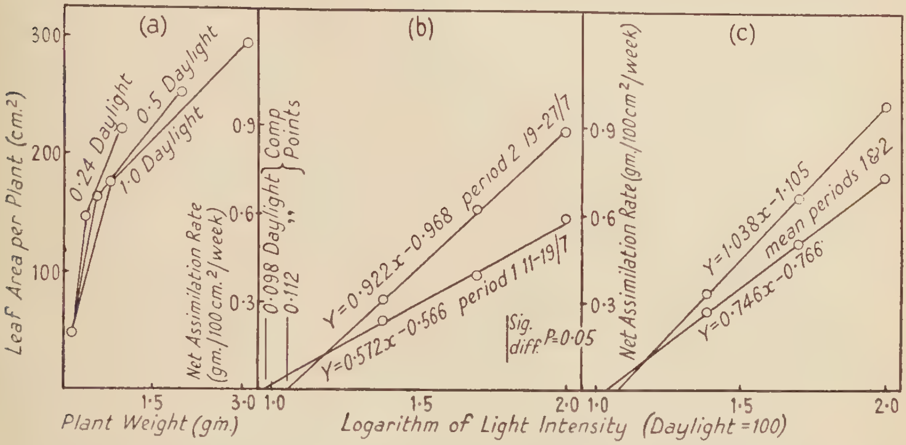


FIG. 7. Expt. 7. *Helianthus annuus.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions, (*b*) net assimilation rate, (*c*) the errors introduced into estimates of net assimilation rate when changes in plant weight and leaf area are not linearly related. Divergence between mean regression of first and second period and regression based on net assimilation rate values calculated from data of initial and final samples.

It should, however, be emphasized that the over-estimation of the net assimilation rates has not resulted in a deviation from the logarithmic relationship with light intensity (see Fig. 7 (*b*) and (*c*)). It is presumed that the errors arising from the non-linearity of the area–weight relationship are proportional to the light intensities and increase at the higher intensities. A further consequence of this proportionate effect is that only minor errors are introduced into the estimation of the compensation point value (see Fig. 7 (*c*)).

In the last of the pot experiments in which *H. annuus* was grown alone there were only two sampling occasions, and over the experimental period the net assimilation rate is proportional to the logarithm of the light intensity (see Fig. 8).

These results for the eight experiments fully confirm the findings of Blackman and Rutter (1948) that net assimilation rate is linearly proportional to the logarithm of decreasing light intensity. Clearly, such a relationship holds, irrespective of the value of the net assimilation rate in full daylight which varied from 0·98 in expt. 4 (Fig. 4) to 0·35 in expt. 2 (Fig. 2). There is a similar fluctuation in the compensation point values from 0·044 daylight in expt. 6 to 0·112 daylight in expt. 7.

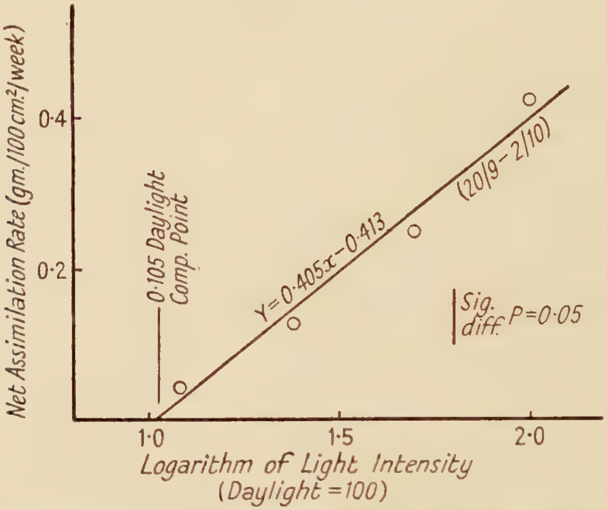


FIG. 8. Expt. 8. *Helianthus annuus.* The effects of varying light intensity on net assimilation rate.

Clearly these variations in assimilation rate must be due to changes in the environmental conditions, since apart from expt. 7, the errors due to the non-linearity of the leaf area–plant weight ratio were small and on the evidence of expt. 1, the differences between experiments cannot be ascribed to age effects.

II. *The effects of varying light intensity on the net assimilation rate of* Helianthus annuus *at different periods of the year*

During the period 1940–3 some eleven field experiments were carried out at Slough. The experiments were basically similar in design since the same types of screens were used throughout, but in each experiment there were only two sampling occasions. As far as the pressure of other war-time research would allow, the individual experiments were initiated at different times of the year so that the effects of season could be assessed. In consequence, during the three years the experimental periods ranged from May 18–29 to October 23–November 11.

From Figs. 9 to 11 it is again evident that net assimilation rate is closely linked with the logarithm of light intensity. Between the nine experimental periods there were major differences in day-length, mean diurnal intensity, and temperature, and these are reflected in the variation in net assimilation rate. Nevertheless, it is immaterial whether the net assimilation rate in full daylight is low as in expt. 10 (0·295), or high, as in expt. 9 (0·763), shading still causes a proportionate logarithmic decrease in assimilation rate. Again in spite of the differences in temperature and light conditions between experiments, the values of the compensation point fluctuate between the relative narrow limits of 0·032–0·063 of daylight.

All the experiments so far considered were carried out at a time when comparatively rapid growth was taking place, for even in expt. 10 the rate of increase in weight per day was 4·8 per cent. In order, therefore, to investigate the effects of shading when conditions were unfavourable to rapid growth, two further experiments were undertaken in which seed was sown late in the autumn and the first sample taken at the end of October. From Fig. 12 it is apparent that the logarithmic relationship between light intensity and net assimilation rate no longer holds. In fact the assimilation rates are higher at the lower light intensities. From these results it is concluded that temperature rather than light intensity is the factor controlling growth at this period of the year and that the protection from night frosts provided by the screens more than offsets the possible effects of a reduction of light intensity on the net assimilation rate.

III. *The effects of varying light intensity on the net assimilation rate of different species*

Although the sunflower has been most frequently employed as the test plant, further experiments have been carried out to determine whether the relationship between the logarithm of light intensity and net assimilation rate is also linear for other species. In some cases the effects have been studied over consecutive periods; in others the results are restricted to a single period.

(*a*) *Fagopyrum esculentum.* The influence of varying light intensity on the net assimilation rate of *F. esculentum* is shown in Figs. 13 and 14. In the first place it is evident that in both expts. 20 and 21 the increase in leaf area at each light intensity is proportional to the change in plant weight and that in consequence no errors associated with a non-linear relationship are involved in the estimations of net assimilation rate. In expt. 20, although the data for 0·67 daylight are below the regression lines, the departures are not significant. Clearly in expt. 21 (Fig. 13) the deviations of the separate period observations are greater than usual, but they are nevertheless not significant. Possibly the slight ground frosts which occurred during the first experimental period may have been an adverse factor. In neither experiment do the mean values for the two periods differ significantly. The fluctuations in the value of the compensation point from 0·06 to 0·099 daylight are smaller than the variations in the assimilation rates in full daylight, i.e. 0·227–0·424.

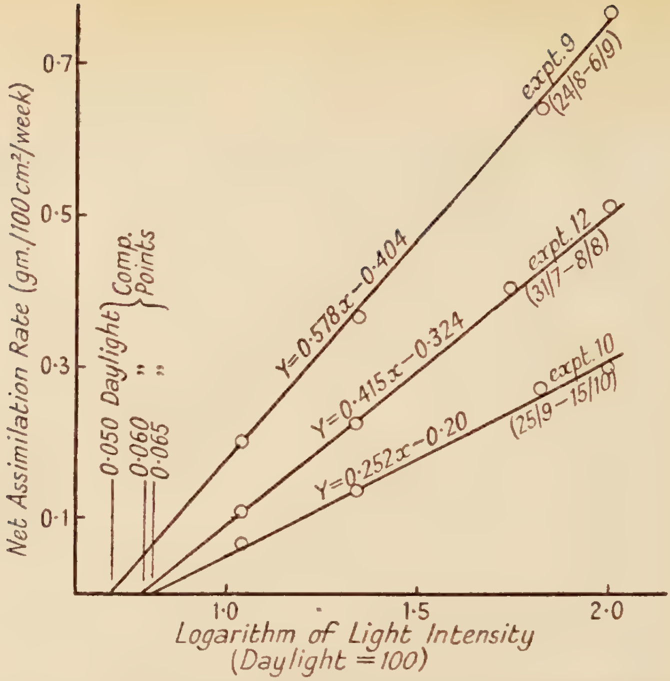


FIG. 9. Expts. 9, 10, 12. *Helianthus annuus.* The effects of varying light intensity on the net assimilation rate at different periods of the year (August–October).

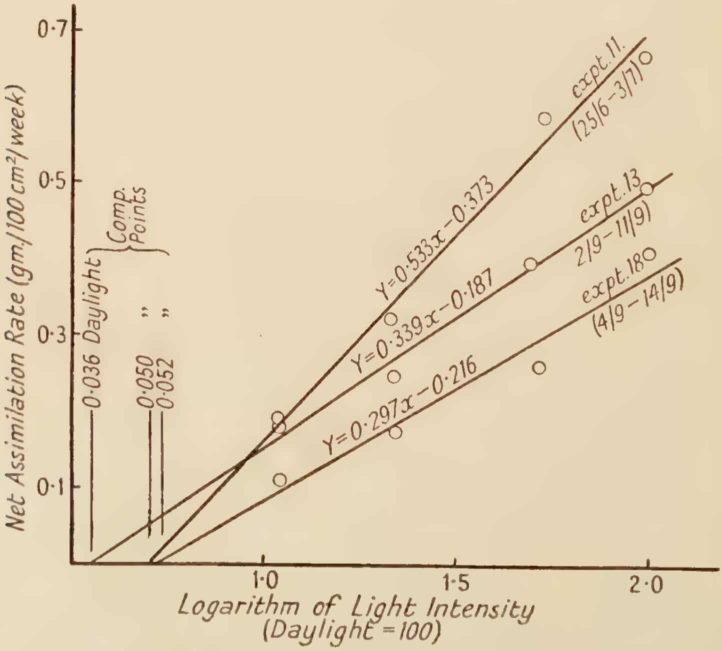


FIG. 10. Expts. 11, 13, 18. *Helianthus annuus.* The effects of varying light intensity on the net assimilation rate at different periods of the year (June–September).

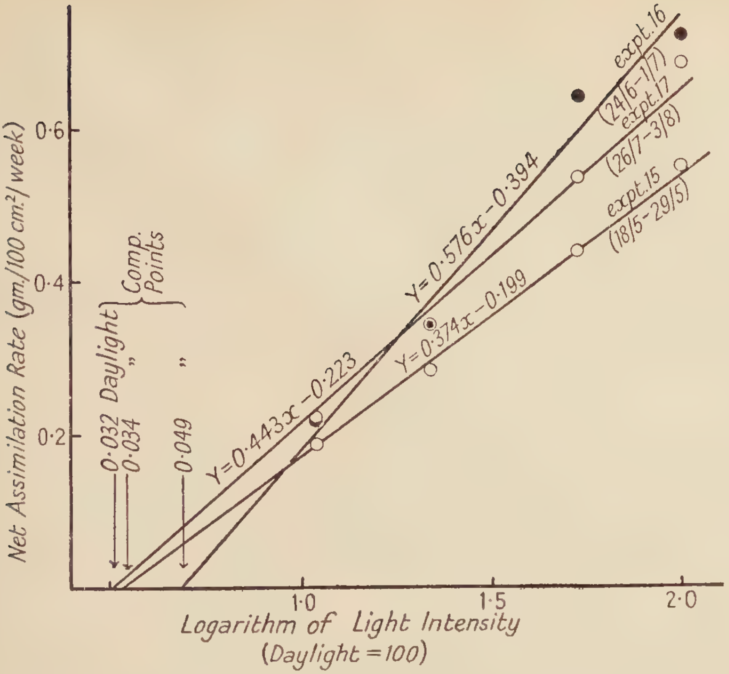


FIG. 11. Expts. 15, 16, 17. *Helianthus annuus.* The effects of varying light intensity on the net assimilation rate at different periods of the year (May–August).

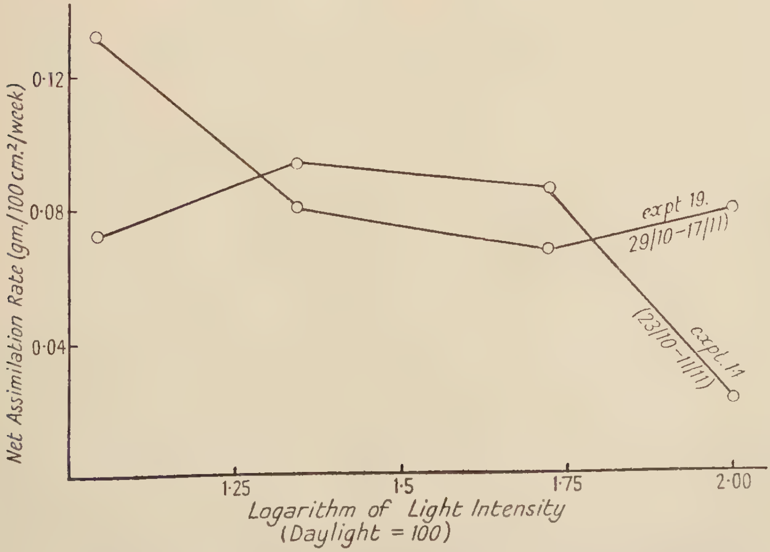


FIG. 12. Expts. 14, 19. *Helianthus annuus.* The effects of varying light intensity on the net assimilation rate in the late autumn (October–November).

(*b*) *Trifolium subterraneum.* The single experiment with *T. subterraneum* again covered two successive experimental periods and the results are given in Fig. 15. Once more it is evident that the relationship between leaf area and plant weight is linear and that the net assimilation rate is logarithmically proportional to the light intensity, although it is apparent that the fit of the regression for the second period is not good. The general level of assimilation for the second period is significantly higher than in the first, while the value of the compensation point is lower.

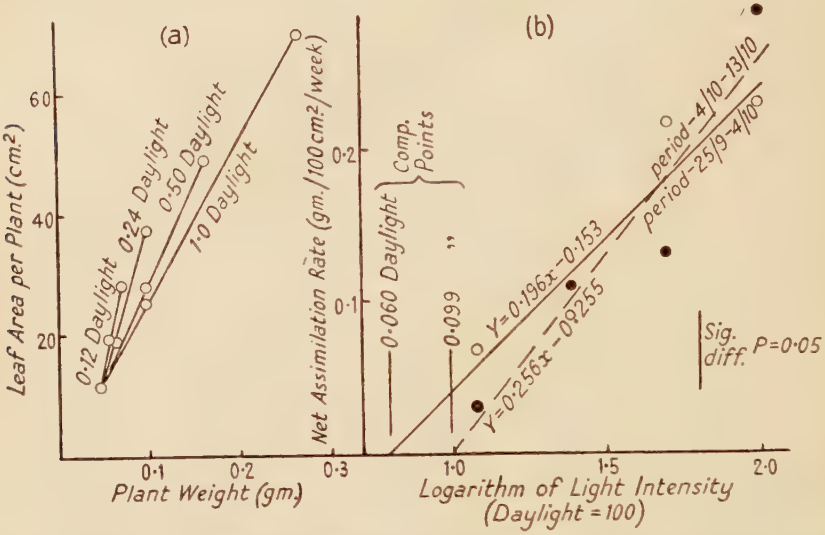


FIG. 13. Expt. 20. *Fagopyrum esculentum.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions and (*b*) net assimilation rate.

(*c*) *Tropaeolum majus.* The experiment employing *T. majus* was carried out somewhat late in the season, and this probably accounts for the low rates of assimilation (see Fig. 16 (*a*)). Nevertheless there is an approximate linear relationship between net assimilation rate and the logarithm of the light intensity.

(*d*) *Lycopersicum esculentum.* Inspection of Fig. 16 (*b*) shows that the regression of net assimilation rate on the logarithm of the light intensity closely fits the experimental data. The results do not support the conclusion of Went (1944) that the maximum assimilation rate of the tomato takes place at light intensities below that of full daylight.

(*e*) *Solanum dulcamara.* Although *S. dulcamara* cannot be regarded as a typical shade plant, nevertheless it germinates in the shade of other plants on to which it eventually climbs. It is therefore of some interest to observe in Fig. 16 (*c*) that the effects of shading in reducing the net assimilation rate are in no way different from those found for other species. Nor is the value of 0·07 for the compensation point exceptional. It must, however, be pointed

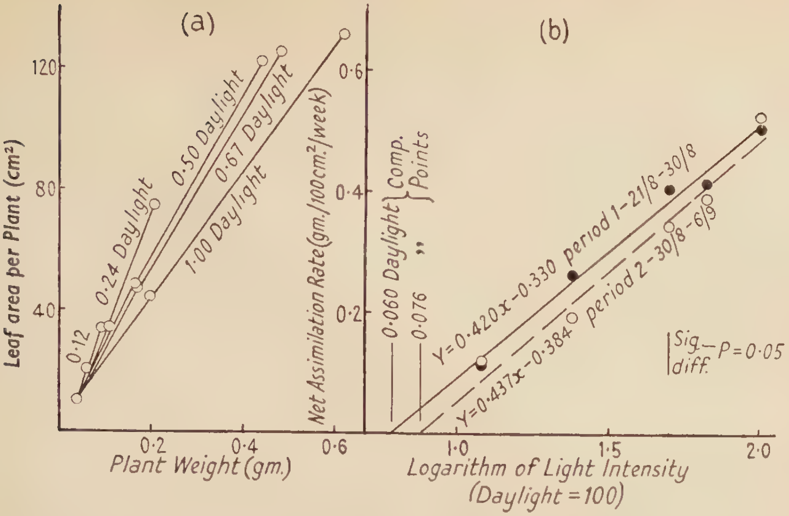


FIG. 14. Expt. 21. *Fagopyrum esculentum.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions and (*b*) net assimilation rate.

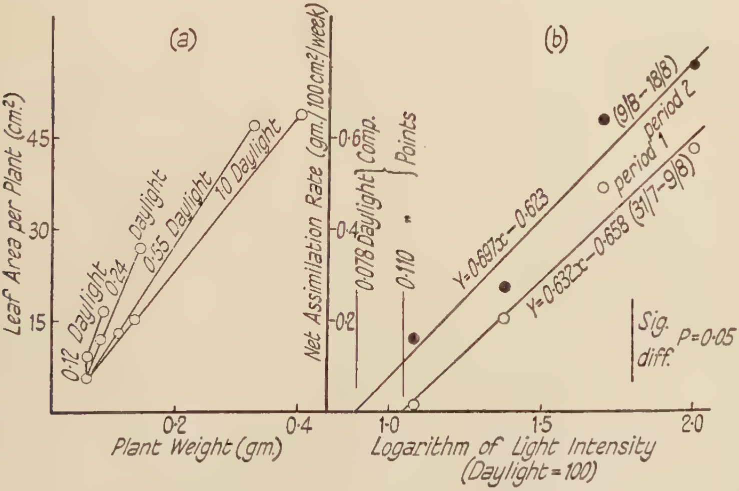


FIG. 15. Expt. 22. *Trifolium subterraneum.* Varying light intensity and (*a*) the relative changes in leaf area and plant weight at successive sampling occasions and (*b*) net assimilation rate.

out that during the experiment there were slight ground frosts, the rate of growth was slow, and the experimental period was considerably longer than usual.

(*f*) *Geum urbanum.* This species has been classed by Lundegardh (1931) as an obligate shade plant, and it would therefore be expected that under conditions of light shade the net assimilation rate should be at least equal to that in full daylight. The evidence of Fig. 16 (*d*) is in direct conflict with this expectation, for it is clear that the net assimilation rate is closely related to the logarithm of the light intensity. Moreover, the compensation point falls within the range of values found for the other species which must come within Lundegardh's definition of sun plants.

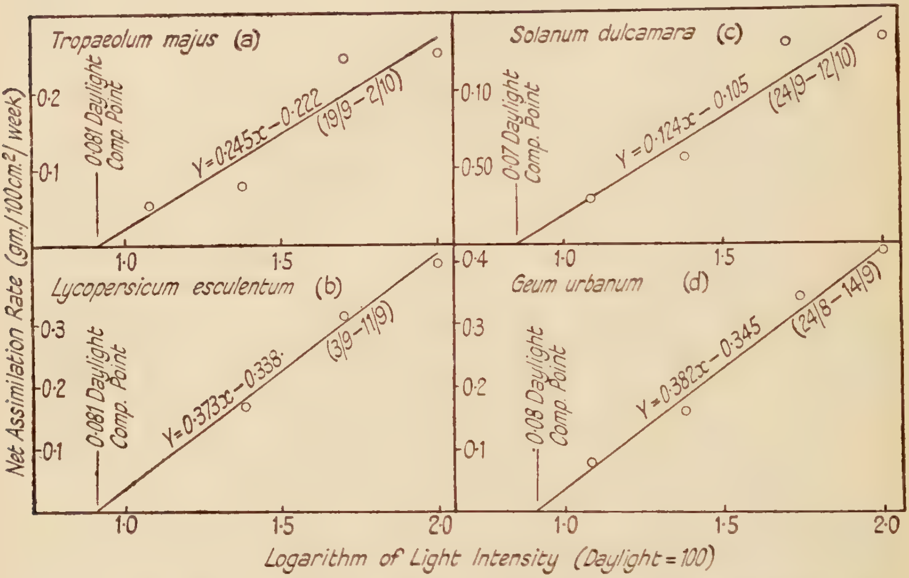


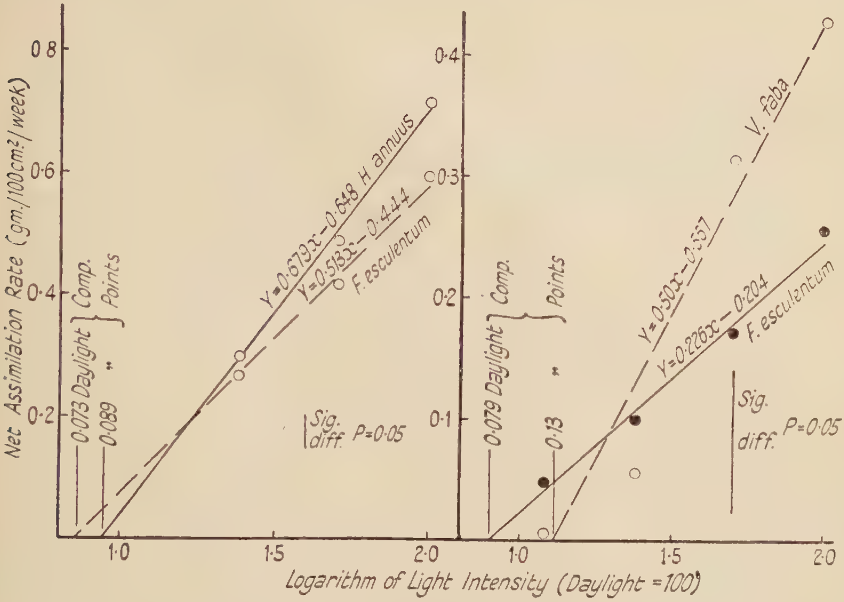
FIG. 16. Expts. 23–6. The effects of varying light intensity on the net assimilation rate of (*a*) *Tropaeolum majus*, (*b*) *Lycopersicum esculentum*, (*c*) *Solanum dulcamara*, (*d*) *Geum urbanum*.

IV. *The comparative effects of varying light intensity on the net assimilation rate of* Fagopyrum esculentum *and other species*

Although the evidence of the previous sections indicates that the relationship between net assimilation rate and light intensity is similar for all species, precise information cannot be obtained without a simultaneous comparison of the reactions of different species. In a number of experiments the results of such a direct comparison are available and in each experiment one of the species was *F. esculentum*.

(*a*) *Fagopyrum esculentum* and *Helianthus annuus*. A comparison of these two species was carried out in mid-August when conditions were favourable for rapid growth. From Fig. 17 (*a*) it is apparent that for both species there is a close linear relationship between the logarithm of light intensity and net assimilation rate. On average the assimilation rate for *H. annuus* is higher and there is a significant interaction of light intensity with species. The values for the compensation point exhibit little difference.

(*b*) *Fagopyrum esculentum* and *Vicia faba*. The results shown in Fig. 17 (*b*) demonstrate that under the experimental conditions the level of assimilation of *V. faba* is markedly greater than that for *F. esculentum*. Since, however, the comparison was not made until relatively late in the autumn when the temperature was low and there were slight ground frosts, it does not follow that such differences would persist at higher temperatures and higher light intensities.



(*a*) (*b*)

FIG. 17. The effects of varying light intensity on the net assimilation rate of (*a*) *Helianthus annuus* and *Fagopyrum esculentum* (expt. 27) and (*b*) *Vicia faba* and *F. esculentum* (expt. 28).

(*c*) *Fagopyrum esculentum*, *Hordeum vulgare*, and *Helianthus annuus*. In this experiment, whereas the data for *F. esculentum* and *H. vulgare* are strictly comparable, a reservation must be made in the case of *H. annuus* since the results are taken from another experiment in which, though the sampling dates were the same, a different soil-sand mixture was employed. It is highly unlikely that this change will have influenced the rate of assimilation, but there is nevertheless no direct proof.

Bearing this qualification in mind, inspecting of Fig. 18 reveals that there is a significant interaction between light intensity and species. Whereas in full daylight the net assimilation rates of buckwheat and barley are higher than that of the sunflower, at the lowest light intensity, barley has the lowest assimilation rate and buckwheat the highest. In consequence, the value of 0·178 daylight for the compensation point of *H. vulgare* is considerably greater than the figures for the other two species. In fact on the basis of these experimental results barley is more sensitive to a reduction in light intensity than any other of the species so far investigated.

(*d*) *Fagopyrum esculentum* and *Pisum sativum.* This experiment was carried out during a period of heavy rainfall and in consequence the pots became waterlogged. The interpretation of the results must therefore take into account a possible differential effect of this waterlogging on the growth of *F. esculentum* and *P. sativum.* On general agronomic grounds it would be expected that the peas would be more adversely affected by excess water and this expectation is in keeping with the experimental results. For example, it is seen in Fig. 19 that while in the case of buckwheat there is, over the whole experimental period, an approximate linear relationship between leaf area and plant weight, yet with peas the area-weight ratios, particularly at the lower light intensities, change between the first and second experimental period. Again, while the regressions of net assimilation rate on the logarithm of light intensity are not significantly different in the case of *F. esculentum*, there is for *P. sativum* a significant effect of occasion. It is not therefore possible to assess precisely whether the variations in net assimilation rate between the two species would have persisted without the conditions of waterlogging. However, since the drainage of the pots was not seriously impeded until half-way through the experiment, it can be tentatively concluded that buckwheat has a higher assimilation rate than the pea.

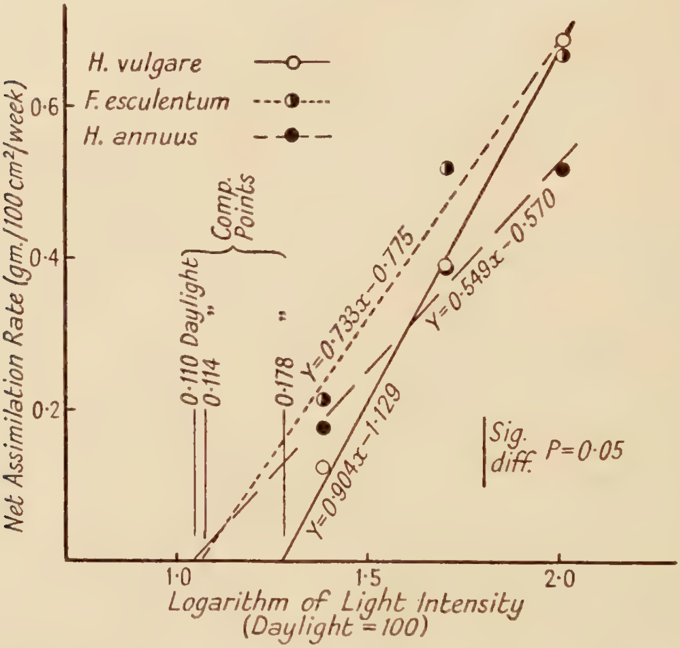


FIG. 18. Expt. 29. The effects of varying light intensity on the net assimilation rate of *Fagopyrum esculentum, Hordeum vulgare,* and *Helianthus annuus.*

DISCUSSION

The results of the present investigation fully confirm that the logarithmic relationship between net assimilation rate and light intensity, first established for *Scilla non-scripta*, is in no way exceptional. In fact, in view of the consistency of the evidence of a similar reaction for the ten other species so far studied, it can be postulated that this logarithmic relationship is of general ecological significance. It is not inferred that there may not on occasion be small but significant departures from linearity, for in some experiments the data do not closely fit the regressions (*vide* Figs. 13, 15, 16 (*c*) and (*d*), 17), and although the discrepancies are not significant, the experimental errors are too large to allow of the detection of such small differences. On the other hand, in none of the other experiments where the errors are relatively low does the departure from linearity approach significance.

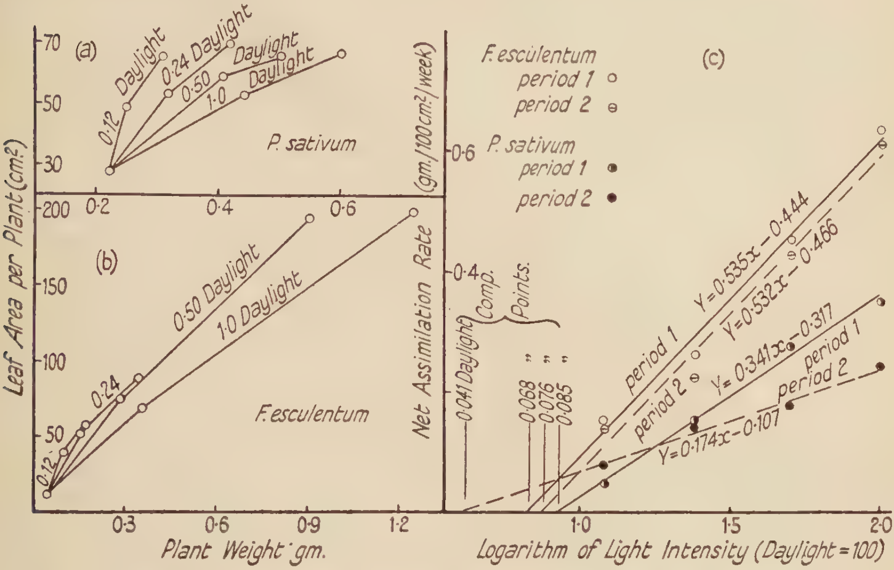


FIG. 19. Expt. 30. Varying light intensity and the relative changes in leaf area and plant weight at successive sampling occasions of (*a*) *Pisum sativum*, (*b*) *Fagopyrum esculentum*, (*c*) varying light intensity and net assimilation rate.

Since there is a common logarithmic relationship between light intensity and net assimilation rate between *G. urbanum*, *S. dulcamara* (Fig. 16), and *S. non-scripta* (Blackman and Rutter, 1948) and the remaining seven species, the concept requires re-examination that plants can be divided into obligate shade plants and sun plants on the basis of a differential reaction of assimilation rate with rising light intensity. Although Lundegardh (1931) classes *G. urbanum* as an obligate shade plant, most of his experimental work was carried out with other woodland plants such as *Oxalis acetosella*, *Melandrium rubrum*, and *Circaea alpina*, the leaves of which according to his investigations reach a maximum assimilation rate at approximately 0·1 daylight. In contrast the leaves of sun plants such as spinach, potato, and *Nasturtium palustre* did not approach a maximum rate until one-third daylight was reached, while above this level the influence of changes in light intensity were negligible. Johansson (1923), Kostytschew and Kardo-Syssoiewa (1930), and Nutman (1937) working respectively with shade ferns, desert plants, and coffee all found that light intensities approaching full daylight either decreased or did not raise the assimilation rate, while Singh and Kumar (1935) concluded that the assimilation rate of radish leaves increased up to three-quarters daylight, thereafter remaining constant.

Thus at first sight it would appear that the findings of these workers are diametrically opposed to the results of the present investigation. It must, however, be emphasized that a close parallel cannot be drawn between carbon dioxide exchange determinations of leaves occupying a few hours and net assimilation rates of whole plants for periods of several days. Comparisons must be even more cautious when these carbon dioxide measurements are not carried out with a high rate of gas flow to ensure that carbon dioxide supply is not limiting assimilation (Heath and Gregory, 1938). Moreover, the effects of a wide range of light intensities on the assimilation of leaves taken from a plant growing in a single light environment are not comparable to the net assimilation rates of plants which have become adapted to varying levels of shade. It has already been demonstrated for *S. non-scripta* (Blackman and Rutter, 1947, 1948), and it will be demonstrated for other species that decreasing the light intensity below full daylight increases the ratio of leaf area to plant weight, the ratio of leaf area to leaf weight, and the proportion of stem, while shading markedly depresses the proportion of root and all these changes will inevitably affect the net assimilation rate. So far no detailed comparison appears to have been made of the effects of varying light intensity on the assimilation of leaves taken from a series of plants fully adapted to a range of light intensities, and until this has been done the effects of shading on net assimilation rate cannot be precisely correlated with changes in the assimilation of detached leaves. In the meantime, in the ecological assessment of the light factor, data on net assimilation rate will possess a greater validity.

The data of Oxley and Ashby (1935) for *Lemna minor* are, however, relevant, since they investigated the effects of light intensity on net assimilation rate and reached the conclusion that this is directly proportional to the light intensity. If, however, their data are plotted against the logarithm of light intensity there is an improved linear relationship over a greater range of intensities. Later investigations by White (1937) with the same species do show a good linearity between net assimilation rate and very low light intensities, but it is doubtful whether this linearity holds over a wider range of intensities, for the value of the compensation point obtained by extrapolation falls far below zero intensity. Moreover, it should be noted that the logarithmic curves of net assimilation rate against light intensity which could be obtained in the present experiments are very nearly linear at the lower light intensities.

Apart, however, from the uniformity of the effects of light on net assimilation rate between species, there is an equal uniformity within species in that the logarithmic relationship holds irrespective of the magnitude of the rate in full daylight, except where the rate is very low in the late autumn (Figs. 11 and 12). For example, in the case of *H. annuus*, the value in full daylight may be as high as 0·98 (Fig. 4) or as low as 0·3 (Fig. 9). Again, with *F. esculentum* this logarithmic response to falling light intensity is common to experiments where the assimilation rate in full daylight varies from 0·66 (Fig. 18) to 0·23 (Fig. 13).

In any consideration of the factors which contribute to the independency of this relationship it is necessary to examine what are the main factors which determine the variations in net assimilation rate. It is clear from Figs. 4, 13, and 14 that between sampling occasions the differences may be small, while, on the other hand, they may equally well be considerable (Figs. 2, 3, 5, 6, 7, and 15). On the evidence of expt. 1 (Fig. 1) such changes cannot be ascribed to ontogenetic drifts in assimilation rate and must therefore be attributed to a change of environmental conditions, such as variations in temperature and mean light intensity.

The influence of temperature of net assimilation rate has been investigated by a number of workers. According to Williams (1939) net assimilation rate, calculated on a leaf protein basis, is closely linked with temperature, while Watson (1947) concluded from analysis of the seasonal growth of several species that there is a positive correlation with temperature. Bolas, Melville, and Selman (1938), measuring the net assimilation rate of tomatoes over short periods of daylight, also found a positive correlation, while Goodall (1945) carrying out similar experiments over periods of several days concluded that when other variables were eliminated only light intensity was significantly linked with net assimilation rate. Ashby and Oxley (1935) in their studies of *Lemna minor* demonstrated that between 18° and 29° C. net assimilation rate was independent of temperatures but that the rate was depressed at 10° C. Similar conclusions were reached by Went (1944) for the tomato when stem elongation in darkness was taken as a measure of the assimilation in the previous light period. At 8° C. assimilation was reduced, but between 17° and 20° C. the change in rate was small.

In view of these somewhat divergent results, an attempt was made to assess the importance of the temperature factor. In the absence of greenhouses with controlled temperatures all that could be done was to grow some pots within the greenhouse at a high temperature and other pots outside under a range of screens. The net assimilation rates in the greenhouse could then be plotted at the appropriate light intensity—*circa* 0·7 of daylight—on a graph in which the net assimilation rate for the outdoor plants had been drawn. The deviation from the regression gives an indication of the 'greenhouse effect' which is largely attributed to temperature, though other factors may be of some importance.

The results of the several experiments are shown in Fig. 20. In the case of tomato, buckwheat, and one of the sunflower experiments, no attempt was made to shade the pots within the greenhouse and so there is only the single light intensity of 0·70 daylight for comparison. Taking first *F. esculentum*, it would appear that within the greenhouse the net assimilation rate has been depressed (Fig. 20 (*b*)), while in the case of the tomato and *H. annuus* it has

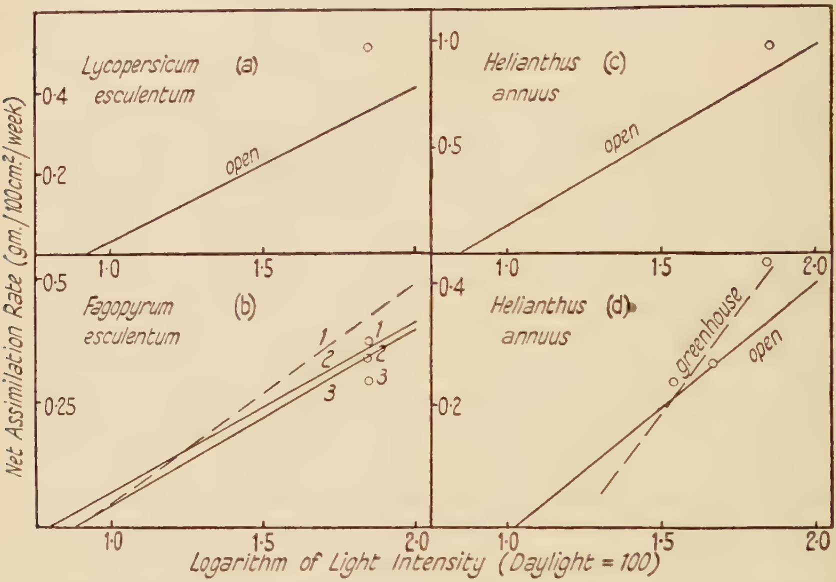


FIG. 20. The differences in the net assimilation of plants grown in a greenhouse and outside (*a*) *Lycopersicum esculentum*, (*b*) *Fagopyrum esculentum*, (*c*) and (*d*) *Helianthus annuus*. In Fig. 20 (*a*), (*b*), and (*c*) the relationships between net assimilation rate and the logarithm of the light intensity in the open are shown as regression lines; the light intensity in the greenhouse was 0·7 daylight and the values of the net assimilation rate are shown as small circles.

been increased. From Fig. 20 (*d*), where there were three levels of light intensity within the greenhouse, it seems that the greenhouse effect is positive at the higher light intensities but less in evidence as the degree of shading is increased.

Unfortunately, except for Fig. 20 (*d*), reliable estimates of the temperature differences within and without the greenhouse are not available, for it was found that the newly installed automatic temperature recorder was not adequately screened from direct insolation. With additional screening there was a mean difference over the experimental period of 5° C. for expt. 8 (Fig. 20 (*d*)), while it is estimated that for expt. 4 (Fig. 20 (*c*)) the difference was 8° C. As might be expected, the differences were greatest during the daytime.

Although these experiments lack precision and must clearly be regarded as of a preliminary nature, they nevertheless indicate that when the maximum day temperature in the open is of the order of 17°–20° C. and the night temperature 10°–12°, then a further rise tends to depress the net assimilation rate of *F. esculentum*. On the other hand, the results for *L. esculentum* and *H. annuus* suggest that at the higher light intensities assimilation rate is positively correlated with temperature. However, when in the several sunflower experiments a comparison is made between the changes in the net assimilation rate of the sunflower over consecutive periods with the corresponding changes in the temperature, the evidence does not point to a marked correlation. In fact from the results in Table I no general conclusion can be drawn. Small differences in temperature between the first and second period may bring about completely opposite effects. Slightly warmer nights and slightly cooler days may lead either to an increase in the net assimilation rate (expt. 7) or to a decrease (expt. 2). A general rise in temperature may raise the assimilation rate (expt. 5) or have little effect (expt. 4).

TABLE I

Variations in Net Assimilation Rate of Helianthus annuus *between Consecutive Periods in relation to Fluctuations in Temperature*

	Net assimilation rate g./100cm.^{2}/week.	Mean daily minimum °C.	Mean daily maximum °C.
Expt. 2			
Period 1 . .	0·58	12·6	22·2
,, 2 . .	0·35*	13·2	21·8
Expt. 3			
Period 1 . .	0·80	14·4	26·5
,, 2 . .	0·54*	11·6	25·2
Expt. 4			
Period 1 . .	0·98	12·1	20·0
,, 2 . .	0·84	14·2	27·0
Expt. 5			
Period 1 . .	0·60*	4·5	14·7
,, 2 . .	0·80	6·8	18·3
Expt. 7			
Period 1 . .	0·58	13·6	24·6
,, 2 . .	0·87*	14·8	23·6

* Differences significant ($P = 0·05$).

On the basis of this evidence it is unlikely that the small changes in temperature due to the screens can have any significance. Measurements of the air temperature made under the screens with a specially constructed whirling thermometer never showed a difference greater than 0·5° C. from the temperature in the open. Further measurements of the soil temperature at a depth of 4 and 8 in. demonstrated that even in the sunniest periods the soil temperatures of the pots in full daylight were never more than 1·5° C. higher than those of the pots receiving 0·24 daylight, while the differences were even less at 0·5 daylight.

If temperature is not a major controlling factor, it can alternatively be advanced that the fluctuations in net assimilation rate are due to changes in light intensity. Since no means of recording and integrating the light intensity was available, a precise relationship between net assimilation rate and total light energy has not been established. For the data of the Oxford sunflower experiments there is a significant correlation with both day-length ($r=0{\cdot}527$) and hours of sunshine ($r=0{\cdot}515$), whereas the correlations with daily minimum, maximum, and mean temperatures are all not significant.

If net assimilation rate is linked with light intensity, it would be expected that the rate would follow the same seasonal trend as the change in the logarithm of the total light energy received per day. Information on the daily change in light intensity from dawn to dusk throughout the year is not readily available and, moreover, the measurements have mostly been made by means of a Callender recorder which has the disadvantage that the infra-red band is included in the integration. However, data derived from measurements employing phototronic cells have been supplied by the National Physical Laboratory for the mean monthly diurnal change, based on a 10-year average at Teddington. From the data the mean energy per day for each month has been estimated in terms of foot candle-hours. These monthly values on a logarithmic scale, together with the observed net assimilation rates in full daylight of all the sunflower and buckwheat experiments, are shown in Fig. 21. It is apparent that although within each month there are considerable variations in net assimilation rate, more particularly in the case of *H. annuus*, the general trend follows the seasonal change in total light energy. Too much cannot be read into the comparisons because, although the sunflower experiments cover 5 years, there were two experimental sites at Slough and Oxford and the changes in light energy throughout the year may differ in some degree from those at Teddington. If, however, light is the major factor controlling net assimilation rate on a logarithmic basis, then it would be anticipated that the mean rate would not change appreciably between May and August, since the diurnal light energy rises only from 25,700 foot candle-hours in May to 30,000 in July and back to 25,900 in August. It would also follow that any major fluctuations in net assimilation rate during the summer would be due to the variations in intensity caused by changes from fine to cloudy weather. With reference to the fluctuations in Fig. 21, it has been calculated on the mean regression derived from the sunflower experiments that the difference between the highest and lowest values of the assimilation rate over the May–August period can be accounted in terms of light intensity if the mean light energy received over the experimental periods varied by the not unlikely ratio of one to four.

Although within species the variation in net assimilation rate may be linked primarily with light intensity and secondarily with temperature, there is evidence that between species these and other factors may operate differentially. In expt. 30 (Fig. 19) it has been recorded that there is a significant interaction between species and occasion in the regressions linking light intensity and assimilation and the greater disparity between *F. esculentum* and *P. sativum* during the second period has been attributed to the waterlogging of the pots being inimical to the pea. Again it has been put forward that the difference in the net assimilation rates of the bean and buckwheat in expt. 28 (Fig. 17 (*b*)) may be due to a differential effect of the cool autumn nights on the two species, since it is well known that *F. esculentum* is sensitive to frost and *V. faba* is not.

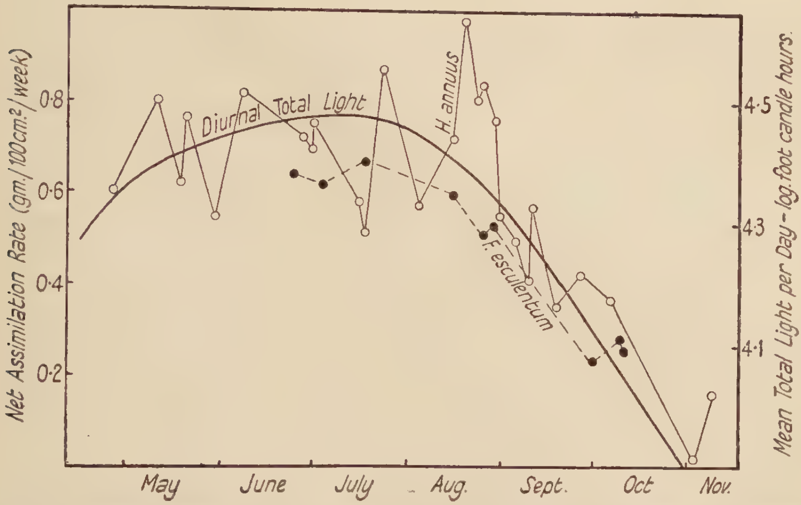


FIG. 21. Seasonal changes in the net assimilation rates of *Helianthus annuus* and *Fagopyrum esculentum* together with changes in the total light per day (10-year average) plotted on a logarithmic scale.

Heath and Gregory (1938), reviewing the then available information, concluded that there is in the open no great variability in the net assimilation rate either between species or between latitudes. Subsequently Watson (1947) showed that significant differences did, in fact, occur between species and even between varieties. The present experiments also point to specific differences (e.g. Fig. 17 (*a*)) in the net assimilation rate, especially where the conditions may be more adverse to one of the species. It is, however, noteworthy that between May and August and when conditions were favourable to rapid growth the range of assimilation rate for *H. annuus* was 0·52–0·98, for *F. esculentum* 0·51–0·67, for *T. subterraneum* 0·58–0·76, and for *H. vulgare* 0·68. In other words, when conditions for growth are optimal, although there may be differences between species, the net assimilation rates are of the same order.

At the other end of the light scale, it is equally clear that the values of the compensation point do not differ widely between species. The mean values for all the species employed in the present experiments, together with the corresponding figure for the earlier *S. non-scripta* investigation (Blackman and Rutter, 1948), are set out in Table II. Apart from *V. faba* and *H. vulgare*, there is a remarkably close agreement between the remaining species. There is little indication from this aspect that the species from shady habitats (*S. non-scripta*, *G. urbanum*, *S. dulcamara*) are any more tolerant of deep shade than other species of open situations. All that these results indicate in the way of specific differences are that barley and the bean demand a higher level of light intensity for assimilation to balance respiration.

TABLE II

The Variation in the Value of the Compensation Point for Different Species

Species.	Mean compensation point (full daylight = 1·0).
Helianthus annuus	0·070*
Fagopyrum esculentum	0·078
Trifolium subteranneum	0·094
Solanum dulcamara	0·070
Geum urbanum	0·080
Pisum sativum	0·063*
Lycopersicum esculentum	0·081
Tropaeolum majus	0·081
Vicia faba	0·136
Hordum vulgare	0·178
Scilla non-scripta	0·089

* Excluding the values for the periods when waterlogging was severe.

These results on general ecological grounds would not have been anticipated. It is true that many workers (e.g. Daxer, 1934) have dismissed the concept of a compensation point as having little significance because it was argued that the value fluctuated widely for any species because of changing light intensity. The present experiments do not, however, indicate that there is a significant correlation between the net assimilation rate in full daylight and compensation point. The data are most comprehensive in the case of *H. annuus*, and the lack of any consistent relationship is clear from Fig. 22. In spite, however, of some variation in the compensation point the coefficient of variation for the mean value is only 15·8 per cent., while the corresponding figure for *F. esculentum* is 18 per cent. It is therefore apparent that the compensation point in no way lacks precision for the ecological assessment of the effects of shade.

Since net assimilation rate is largely the balance between the gains due to assimilation by the leaves and the respiration losses of the whole plant, the light factor can operate in several ways. Shading by reducing the light energy reaching the leaves can decrease carbon fixation; the reduced supplies of carbohydrate may affect the respiration of the different parts; the proportions and structure of root stem and leaves will be markedly altered by the level of light intensity. The logarithmic relationship between light intensity and net assimilation rate is therefore an integration of many possible effects and there is as yet an inadequate basis of knowledge on which to judge their relative importance. For example, the relative respiration rates of whole plants or their individual parts grown under sun and shade conditions do not appear to have been investigated. In fact, singularly little information is available on the comparative respiration of the different parts of a plant—the data of Inamdar, Singh, and Pande (1925) are by far the most complete. They found that, in the case of cotton, the leaf and root respiratory losses amounted to 0·07 of their dry weight per day while the figure for the stem was about 0·04. With balsam the daily losses ranged from 0·10 for the stem to 0·2 for the roots. For whole shoots or leaves figures of the same order have been obtained by other investigators. On the other hand, Goodall (1945), Harris (1939), and Woodford and Gregory (1948), working with tomatoes, apples, and barley, estimate that half to two-thirds of the material translocated to the roots is lost in respiration. Moreover, it will be shown in later paper that one of the major effects of shading is to alter the root-shoot ratio and the reduction in the proportion of root may in turn reduce the respiration losses of the whole plant and hence affect the net assimilation rate.

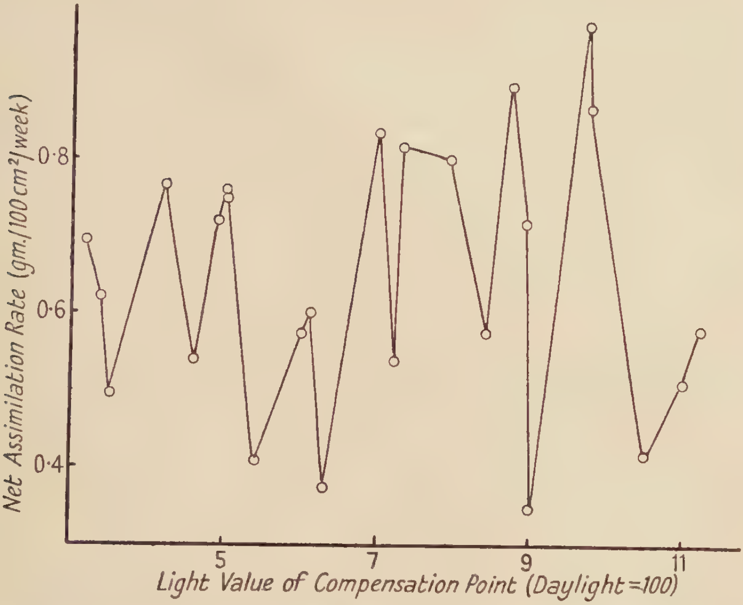


FIG. 22. The lack of correlation between variations in the net assimilation rate of *Helianthus annuus* in full daylight and the corresponding fluctuations in the value of the compensation point.

Although the tentative conclusion has been reached that light intensity rather than temperature is the major factor controlling the net assimilation rate, it does not follow that temperature may not affect the proportions of the plant or the respiration of its individual parts. Evidence will be presented in a later paper that in shaded plants the proportion of stem tends to increase with rising temperature, while on general grounds it would be expected that the levels of respiration would be linked with temperature.

From the foregoing discussion it is apparent that the complexity of the factors governing the logarithmic relationship between light intensity and net assimilation rate will not be clarified without a further series of investigations on the several problems involved. On the other hand, from the viewpoint of the ecological significance of the light factor the present results stress that the major effects of shading on the net assimilation rate are common to a large number of species. The ability of different species to withstand shading cannot be attributed to major variations in either the rate of assimilation at low light intensities or the compensation point. It is not, however, inferred that the relationship between growth rate and varying light intensity is the same for all these species, for it will be shown subsequently that there are specific differences in the relative growth rate under shade conditions and that these differences are linked with varying changes in the leaf area ratio induced by a reduction in the light level.

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The senior author is indebted to A. J. Rutter, J. Carpenter, and C. P. Wittingham for their assistance in carrying out the experiments at Slough.

SUMMARY

In this further study of light as an environmental factor, the effects of shading on the growth of some ten species have been investigated. Over a period of 5 years some thirty field or pot experiments have been carried out in which screens have been employed to provide light intensities ranging from 0·1 to 1·0 of normal daylight. Most attention has been given to *Helianthus annuus* and *Fagopyrum esculentum*, but the following species have also been studied: *Trifolium subterraneum*, *Tropaeolum majus*, *Lycopersicum esculentum*, *Vicia faba*, *Pisum sativum*, *Hordeum vulgare*, *Solanum dulcamara*, and *Geum urbanum*.

All experiments have been undertaken with young plants in the vegetative stage, and the methods of growth analysis have been used to elucidate the effects of shading on net assimilation rate. In the individual experiments two to five samples at intervals of 4 to 21 days have been taken for the determination of the leaf area and the dry weights of the root, stem, and leaves.

It has been established that the *reductions in the net assimilation caused by shading are similar for all ten species*. Irrespective of whether the species are normally associated with shady habitats (e.g. *G. urbanum*) or grow in the open (*H. annuus*, *T. subterraneum*), the *net assimilation rate during the season of active growth is linearly related to the logarithm of the light intensity*. The agreement between the forty-six fitted regressions and the experimental observations is in many cases remarkably good. When similar experiments are conducted

late in the autumn and the relative growth rate is very low the logarithmic relationship no longer holds.

In comparative experiments it has been demonstrated that the regressions linking net assimilation rate and light intensity may differ between species. For *H. annuus* and *F. esculentum* the differences are small. With barley, shading brings about larger reductions in the net assimilation rate than it does with *H. annuus* or *F. esculentum*. Under conditions of waterlogging, the net assimilation rates of *P. sativum* are consistently less at all light intensities than those of *F. esculentum*, and under the cool conditions of September *V. faba* assimilates more than *F. esculentum*.

Both between experiments and between experimental periods there may be large differences in the net assimilation rate. For *H. annuus* over the months of May to September the highest rate in full daylight was 0·98 g. per 100 cm^2 per week and the lowest 0·3. Such differences cannot be attributed to ontogenetic drifts since it was demonstrated that appreciable differences in the age of the plants had no effect on net assimilation rate.

By comparing the net assimilation rates of plants grown in a greenhouse with those grown outside in the open, preliminary evidence was obtained that while the warmer conditions of the greenhouse increased the assimilation rate of *L. esculentum* and *H. annuus* there was little effect on *F. esculentum*. However, the seasonal variations in the net assimilation rate of *H. annuus* in full daylight showed little correlation with temperature, but the correlation coefficients for length of day and hours of sunshine were significant. The data for *H. annuus* and *F. esculentum* (in all thirty-six observations) indicate that between May and August the net assimilation rate does not change appreciably, but falls sharply during September.

From the linear regressions it is possible to obtain precise estimates of the compensation point, and it has been found that *the degree of shading required to reduce the net assimilation rate to zero is similar for all species*. The mean values of the compensation point ranged from 0·06 to 0·09 of daylight for eight species, while for *V. faba* and *H. vulgare* somewhat higher figures were obtained—0·14 and 0·18 of daylight.

Fluctuations in the net assimilation rate in full daylight showed no correlation with variations in the value of the compensation point.

From these results it is concluded that species cannot be grouped into 'sun' or 'shade' plants, either on the basis of differences in the value of the compensation point or on the grounds that there are large variations in the effects of shading on net assimilation rate.

LITERATURE CITED

ASHBY, E., and OXLEY, T. A., 1935. The Interaction of Factors in Growth of *Lemna*. III. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication. Ann. Bot., xlix. 309.

ATKINS, W. R. G., POOLE, H. H., and STANBURY, F. A., 1937. The Measurement of Light Intensity and the Colour of Light in Woods by means of Emission and Rectifier Photoelectric Cells. Proc. Roy. Soc. B, cxxi. 427.

Blackman, G. E., 1946. Sunflowers as an Oil Seed Crop. Journ. Min. Agric., l. 517.

—— and Rutter, A. J., 1946. Physiological and Ecological Studies in the Analysis of Plant Environment. I. The Light Factor and the Distribution of the Bluebell (*Scilla non-scripta*) in Woodland Communities. Ann. Bot., N.S., x. 361.

—— —— 1947. II. The Interaction between Light Intensity and Mineral Nutrient Supply in the Growth and Development of the Bluebell (*Scilla non-scripta*). Ibid., xi. 126.

—— —— 1948. III. The Interaction between Light Intensity and Mineral Nutrient Supply in Leaf Development and in the Net Assimilation Rate of the Bluebell (*Scilla non-scripta*). Ibid., xii. 1.

—— —— 1949. IV. The Interaction between Light Intensity and Mineral Nutrient Supply on the Uptake of Nutrients by the Bluebell (*Scilla non-scripta*). Ibid., xiii. 453.

—— —— 1950. V. An Assessment of the Factors controlling the Distribution of the Bluebell (*Scilla non-scripta*) in Different Communities. Ibid., xiv. 487.

Bolas, B. D., Melville, R., and Selman, I. W. 1938. The Measurement of Assimilation and Translocation in Tomato Seedlings under the Conditions of Greenhouse Culture. Ibid., ii. 717.

Daxer, H., 1934. Über die Assimilations-ökologie der Waldbodenflora. Jahrb. Wiss. Bot., lxxx. 363.

Goodall, D. W., 1945. The Distribution of Weight Change in the Young Tomato Plant. I. Dry Weight Changes of the Various Organs. Ann. Bot., N.S., ix. 101.

Harris, G. H., 1929. Studies on Tree Root Activities. Part II. Some Factors which influence Tree Root Respiration. Sci. Agric., ix. 564.

Heath, O. V. S., and Gregory, F. G., 1938. The Constancy of the Mean Net Assimilation Rate and its Ecological Importance. Ann. Bot., N.S., ii. 811.

Inamdar, R. S., Singh, S. B., and Pande, F. D., 1925. The Growth of the Cotton Plant in India. I. The Relative Growth Rates during Successive Periods of Growth and the Relation between Growth Rate and Respiratory Index throughout the Life Cycle. Ibid., xxxix. 281.

Johansson, N. 1923. Zur Kenntniss der Kohlensaure-assimilation einiger Farne. Svensk. Bot. Tidskrift, xvii. 215.

Kostytschew, S., and Kardo-Syssoiewa, H. 1930. Untersuchungen über den Tageslauf der Photosynthese in Zentralasien. Planta, xi. 117.

Lundegardh, H., 1931. Environment and Plant Development (trans. E. Ashby). Arnold London.

Nutman, F. J., 1937. Studies in the Physiology of *Coffee arabica*. I. Photosynthesis of Coffee Leaves under Natural Conditions. Ann. Bot., N.S., i. 353.

Petrie, A. H. K., and Arthur, J. I., 1943. Physiological Ontogeny in the Tobacco Plant. The Effect of Varying Water Supply on the Drifts in Dry Weight and Leaf Area and on Various Components of the Leaves. Austral. Journ. Exp. Biol. and Med. Sci., xxi. 191.

Singh, B. N., and Kumar, K., 1935. The Reactions of the Assimilatory System to Alterations in Light Intensity. Proc. Indian Acad. Sci., B, i. 754.

Tiver, N. S., 1942. Studies of the Flax Plant. I. Physiology of Growth, Stem Anatomy and Fibre Development in Fibre Flax. Austral. Journ. Exp. Biol. and Med. Sci., xx. 149.

—— and Williams, R. F., 1943. II. The Effect of Artificial Drought on Growth and Oil Production in a Linseed Variety. Ibid., xxi. 201.

Watson, D. J., 1947. Comparative Physiological Studies on the Growth of Field Crops. I. Variations in Net Assimilation Rate and Leaf Area between Species and Varieties, and within and between Years. Ann. Bot., N.S. xi. 41.

Went, F. W., 1944. Plant Growth under Controlled Conditions. III. Correlation between various Physiological Processes and Growth in the Tomato Plant. Amer. Journ. Bot., xxxi. 597.

White, H. L., 1937. The Interaction of Factors in the Growth of *Lemna*. XI. The Interaction of Nitrogen and Light Intensity in Relation to Growth and Assimilation. Ann. Bot., N.S., i. 623.

Williams, R. F., 1937. Drift of Net Assimilation Rate in Plants. Nature, xcl. 1099.

—— 1946. The Physiology of Plant Growth with Special Reference to the Concept of Net Assimilation Rate. Ann. Bot., N.S., x. 41.

Woodford, E. K., and Gregory, F. G., 1948. Preliminary Results obtained with an Apparatus for the Study of Salt Uptake and Root Respiration of Whole Plants. Ibid., xii. 335.

Symbiosis of Leguminous Plants and Nodule Bacteria

IV. The Importance of the Oxygen Factor in Nodule Formation and Function

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With Plate VII and one Figure in the Text

ABSTRACT

Nodulated plants of soya-bean have been grown in culture solutions lacking combined nitrogen and in continuous contact with oxygen/nitrogen gas mixtures containing 21, 12, and 5 per cent. oxygen respectively. Reductions of oxygen content led to reductions in dry weight and in fixation of nitrogen.

Somewhat similar but smaller effects on dry weight were observed with non-nodulated plants supplied with ammonium-nitrogen, but such plants supplied with nitrate-nitrogen grew equally vigorously at all three oxygen levels, a result regarded with some reservation.

The weight of nodules per plant was markedly reduced by curtailment of oxygen, but the number was unaffected, indicating that the earlier stages in nodule formation are not specially sensitive to oxygen supply. The greater effect of oxygen reduction on nodulated plants appears to be exerted on the process of fixation or on associated processes such as nodular respiration.

INTRODUCTION

IN previous work (Bond, 1950) information was sought on the relative importance of oxygen for the development and functioning of leguminous root nodules by means of experiments in which the growth of aerated and non-aerated water cultures of soya-bean was compared in respect of nodulated plants in nitrogen-free solution and non-nodulated plants supplied with nitrate-nitrogen. It was found that the nature of the results varied from experiment to experiment, particularly in respect of the nodulated plants, the growth of which was on some occasions markedly benefited by aeration, while on other occasions there was little effect. It was concluded that this was mainly due to the poor control over the oxygen content of the culture solution in the above type of experiment. It was therefore decided to adopt an improved technique giving closer control over oxygen tension and resembling in some respects that employed by Gilbert and Shive (1942). The technique and the results obtained with it are described in the following sections.

Most of the relevant literature was reviewed previously (Bond, 1950), and certain papers will be considered further later in the present paper. The discovery of the presence of haemoglobin in the leguminous root nodule has

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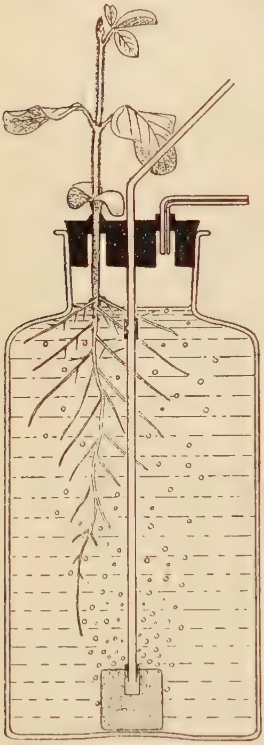
increased interest in the oxygen relation of the nodule, though at present there is little experimental evidence to show how the two are connected (Smith, 1949).

METHODS

The Montreal Manchu variety of soya-bean has been employed in these experiments, the origin and treatment of the seed, and the stage at which young plants were transplanted into non-flowing water culture, being as previously described (Bond, loc. cit.). The glass jars used for water culture had a capacity of 2½ litre, and were 25 cm. in height, with diameters of 6 cm. and 13 cm. at the mouth and below the shoulder respectively. Four of the holes in the rubber stoppers fitted to the jars were of diameter 1 cm., and through three of these the roots of the young plants were introduced and the hypocotyls sealed into the holes by means of modelling clay ('plasticine'). The fourth large hole was closed by a small stopper bearing the gas outlet, consisting of a short length of capillary tubing of 1 mm. bore; by removing this small stopper access could be gained to the culture solution for purposes of renewal of solution or withdrawal of samples. The fifth, smaller hole served for the gas inlet tube which extended to the bottom of the jar and was inserted there into a cubical porous porcelain aerator of 3 cm. edge. These features are shown in the text-figure. A nitrogen-free form of Crone's solution (formula B of previous paper, plus the minor elements indicated there) has been used, with the sediment included. The medium was autoclaved before use, and all fittings to the jars were initially sterilized against the nodule organism.



Details of culture jar (actually there were three plants per jar). × ¼. For description see text.

A few hours after transplanting, the passage of different oxygen/nitrogen gas mixtures (see below) through the jars was commenced, after which certain jars were inoculated with an effective strain of the soya nodule organism (University of Wisconsin No. 505). Other jars received nitrate- or ammonium-nitrogen (as $NaNO_3$ or $(NH_4)_2SO_4$ respectively), but the first addition was delayed for a time in order to compensate for the nitrogen-hunger period experienced by the inoculated plants. Thereafter further amounts of combined nitrogen were added at frequent intervals. A small number of control jars, uninoculated and without added nitrogen, was included in order to confirm that the cultural conditions provided no unintended access to sources of combined nitrogen.

Three levels of oxygen were maintained in the culture jars, the highest being obtained by passing air (21 per cent. oxygen), and two other levels by passing, through other series of jars, oxygen/nitrogen mixtures containing respectively 12 per cent. and 5 per cent. of oxygen.[1] The gas streams, which continued to be passed without serious interruption throughout the experiment, were scrubbed through culture solution similar to that supplied to the plants and were then dried over calcium chloride before passing to the culture jars. It was assumed and was later confirmed by oxygen determinations that the rates of flow of the gas would suffice to prevent the entry of atmospheric oxygen through the exit tube. The rate of flow of the gas currents in the earlier stages of the experiment was of the order of 1 litre per hour per jar, but was increased later in accordance with the results of oxygen determinations (see below), the object being to maintain oxygen tensions in the solutions as nearly as possible at the equilibrium level corresponding to the proportion of oxygen in the gas passed. Naturally more rapid flow was required in the later stages, when roots and nodules were well developed.

The plasticine seals round the hypocotyls were adjusted and tested for gas-tightness daily, and were completely renewed fortnightly. Also at fortnightly intervals in the earlier stages of the experiments, and more frequently later, the culture solution in the jars was replaced by fresh solution, while the level of solution in the jars was restored daily to the neck by the addition of water. The pH in jars of nodulated plants was adjusted every 24 hours to 6·5 by addition of N/10 NaOH, while with plants receiving ammonium-nitrogen this operation was carried out every 12 hours, precautions being taken to avoid the introduction of the nodule organism into the latter jars. No uninoculated plants developed nodules.

Determinations of dissolved oxygen in the culture solution were made by the method previously employed (Bond, 1950), samples being drawn from levels 8 cm. and 17 cm. from the surface of the culture solution, the total depth of which was 21 cm. The results of the determinations have again been expressed as a percentage of the oxygen content of water in equilibrium with air at the temperature and pressure prevailing at the time of the determinations, using the data of Winkler.

The experiments were continued until pods were present on the plants, the arrangement of the jars in the greenhouse being frequently changed. Any leaves falling from the plants were stored and added to the appropriate plant material at harvest. Dry weights were obtained by heating the plant material at 95° C., and total nitrogen by Kjeldahl process.

EXPERIMENTAL RESULTS

Two experiments have been conducted, the first (1948) including nodulated plants and plants in receipt of nitrate-nitrogen, the second experiment (1949) being similar except that ammonium-nitrogen was substituted for nitrate-nitrogen, in view of the possibility that oxygen of the nitrate ion can be utilized by plants as an alternative source of oxygen under conditions of reduced supply of free oxygen (Gilbert and Shive, 1945; Bond, 1950).

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[1] The oxygen/nitrogen mixtures and the air were obtained in the compressed form from The British Oxygen Co., Ltd.

The results of determinations of dissolved oxygen in the two experiments, at the 8-cm. level, are summarized in Tables I and II. Expressed in the

TABLE I

Results of Determinations of Dissolved Oxygen, 1948 Experiment, on Samples taken at a Depth of 8 cm. from Surface of Culture Solution

Type of culture.		Days from sowing.								
		17	24	36	46	55	57	63	81	95
Air	Nod.	98 (2)*	96 (4)	97 (2)	93 (2)	82	82	91	95	80
	Non-nod.†	—	—	—	—	80	100	91	84	79
12% O_2	Nod.	55 (2)	51 (6)	52 (2)	52 (4)	43	34 (2)	53	49	41
	Non-nod.†	—	—	—	45	24	46	—	45	35
5% O_2	Nod.	18 (2)	21 (6)	19 (2)	22 (5)	20	11 (2)	27	21	17
	Non-nod.†	—	—	—	14	6	25	14	15	16

* Oxygen values are expressed as percentages of the oxygen content of water in equilibrium with air (see text). Figures in parentheses indicate the number of individual determinations, on different jars, of which the value shown is the mean. Other values are based on a single determination.

† Plants receiving nitrate-nitrogen.

TABLE II

Results of Determinations of Dissolved Oxygen, 1949 Experiment, on Samples taken at a Depth of 8 cm. from Surface of Culture Solution

Type of culture.		Days from sowing.								
		20	32	38	46	59	62	73	76	85
Air	Nod.	99*	91 (3)	90 (3)	88 (2)	68 (2)	89 (2)	81 (2)	88 (2)	77 (2)
	Non-nod.†	—	—	—	—	87	88	71	86 (2)	—
12% O_2	Nod.	52 (3)	52 (2)	49 (2)	53 (2)	41 (3)	39 (2)	50 (2)	46 (2)	36
	Non-nod.†	—	—	—	—	46	41	47	45 (2)	—
5% O_2	Nod.	21 (3)	21 (2)	19 (2)	15 (2)	15 (2)	15 (2)	15 (2)	16 (2)	15
	Non-nod.†	—	—	—	—	13	13	14	10 (2)	—

* For explanation of method of expression of oxygen data and significance of figures in parentheses see Table I.

† Plants receiving ammonium-nitrogen.

manner indicated on p. 97, the anticipated values for dissolved oxygen corresponding to oxygen/nitrogen mixtures containing 21, 12, and 5 per cent. oxygen are 100, 57, and 24 respectively. The tables show that in the early stages the results of determinations were fairly close to these theoretical values; that the observed values did not exceed the theoretical ones in the case of the two lower levels of oxygen confirms that there was no significant back-entry of atmospheric oxygen via the outlet tube. As the plants increased in size and root and nodule respiration reached greater magnitude, it became increasingly difficult to keep the oxygen tensions up to the desired levels, without employing prodigal rates of gas-flow. The means of all the values shown in Tables I and II are as follows:

	Air.		12% oxygen.		5% oxygen.	
	Nod.	Non-nod.	Nod.	Non-nod.	Nod.	Non-nod.
1948	93	87	49	39	20	15
1949	85	84	47	45	17	12

On several occasions samples for oxygen determinations were drawn from the lower level (17 cm.) as well as from the higher, the data being presented in Table III. Oxygen tensions at the two levels were practically identical in every case, and it may be concluded that tension was satisfactorily uniform throughout the depth of the jars. Further implications of the oxygen data will be considered in the Discussion.

TABLE III

Oxygen Content of Samples of Culture Solution taken from Two Levels in the Same Jars

Type of culture.		Sample level.*	15/7/48.	24/8/48.	4/9/48.	25/7/49.
Air	Nod.	8	93	—	80	77
		17	87	—	80	78
	Non-nod.†	8	—	84	79	—
		17	—	84	86	—
12% O_2	Nod.	8	53	—	41	36
		17	51	—	39	36
	Non-nod.†	8	—	45	35	—
		17	—	42	35	—
5% O_2	Nod.	8	24	—	17	15
		17	21	—	19	15
	Non-nod.†	8	—	15	16	—
		17	—	19	15	—

* Cm. from surface of culture solution. † Plants receiving nitrate-nitrogen.

The harvest data for the nodulated plants of the 1948 experiment are presented in Tables IV and V. Nodules appeared 12 days after inoculation, while definite signs of fixation were obvious approximately 4 weeks after inoculation. The adjustment of pH for nodulated plants was commenced in the fifth week of the experiment, a fall in pH to a value between 4 and 5 frequently occurring during the 24 hours between adjustments. Soon after fixation had commenced a direct relation between the oxygen tension and the general vigour of the nodulated plants became obvious and continued until the end of the experiment. Table IV shows (the use of statistics being unnecessary) that the dry weights of all organs fell progressively as oxygen tension was

TABLE IV

*Data obtained at Harvest of Nodulated Plants of Experiment commenced June 1, 1948**

% oxygen in gas supplied.		No. of nodules.	Dry weights (g.).				Relative total.
			Nodules.	Roots.	Tops.	Total.	
21		1,205	1·16	1.52	7·62	10·30	
		1,315	1·20	1·38	7·31	9·89	
		1,153	1·10	1·60	8·50	11·20	
		1,072	1·25	1·47	8·01	10·73	
		1,151	1·26	1·45	7·59	10·30	
	Means	**1,179**	**1·19**	**1·48**	**7·81**	**10·48**	100
12		902	0·91	1·04	6·33	8·28	
		1,162	1·14	1·38	7·64	10·16	
		985	1·07	1·52	7·05	9·64	
		1,052	0·97	1·17	6·05	8·19	
		1,180	0·85	1·11	5·87	7·83	
	Means	**1,056**	**0·99**	**1·24**	**6·59**	**8·82**	84
5		1,077	0·67	0·82	4·68	6·17	
		1,170	0·53	0·81	3·87	5·21	
		1,233	0·78	0·98	5·27	7·03	
		966	0·77	0·84	5·22	6·83	
		1,143	0·64	0·96	4·30	5·90	
	Means	**1,118**	**0·68**	**0·88**	**4·67**	**6·23**	59

* Data are per jar of three plants, with five jars per treatment. These nodulated plants were harvested September 6.

TABLE V

Data for Mean Total Nitrogen of Plants of Table IV (mg. per jar of three plants)

% oxygen in gas supplied.	Nodules.	Tops and roots.	Whole plants.	Whole plants, relative basis.
21	56	236	292 (15)*	100
12	47	201	248 (11)	85
5	30	94	124 (8)	42

* Figures in parentheses are standard errors of the means. To be significant, differences between the means for whole plants must exceed 36 mg. ($P = 0{\cdot}05$).

lowered. The relative figures bring this out clearly for total dry weight, the effect of the reduction from 12 to 5 per cent. oxygen being particularly marked. Similar statements hold for the nitrogen content and fixation,[1] data for which, with statistical treatment, are summarized in Table V. It will be noted (Table IV) that although the dry weight of nodules fell with diminishing oxygen tension, the number of nodules remained essentially unchanged. Typical plants of the nodulated series are shown in Pl. VII, Fig. 1.

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[1] Eight control jars of non-nodulated plants not supplied with combined nitrogen were grown in connexion with the two experiments described in this paper, with one or more at each oxygen level. The nitrogen content of these plants at harvest ranged from 35 to 42 mg. per jar of three plants, with a mean of 37 mg., which is chiefly ascribable to the original seed nitrogen (32 mg. for three seeds). Deduction of this figure of 37 mg. from the nitrogen contents of nodulated plants in Tables V and VIII indicates the amount of nitrogen fixed.

Data for the plants receiving nitrate-nitrogen of the 1948 experiment are summarized in Table VI. Although the first addition of nitrate was delayed for 3 weeks after transplanting, these plants soon surpassed in growth those dependent on nodule nitrogen and, as will be noted, greatly exceeded them in dry matter at harvest. The pH (which here received no adjustment) tended to rise from that of the freshly supplied solution (6·2), values of up to 7·7 being recorded after 10 days' contact with the roots. The data in Table VI indicate that growth was substantially unaffected by the lowering of oxygen tension, as is confirmed by the photograph (Pl. VII, Fig. 2). It should be noted that the plants absorbed a high proportion of the nitrate-nitrogen supplied, and it is possible that shortage of nitrogen limited growth at some stages. In view, however, of the luxuriant growth of all the plants it is difficult to believe that growth could have been much greater at any particular oxygen level had more nitrate been supplied. It may be noted that Gilbert and Shive (1942; see also Bond, 1950) found dry weights of soya-bean receiving nitrate to be similar with gas-flows containing 20 and 10 per cent. oxygen respectively.

TABLE VI

*Mean Data obtained at Harvest of Non-nodulated Plants receiving Nitrate-Nitrogen of Experiment commenced June 1, 1948**

% oxygen in gas supplied.	Dry weights (g.). Roots.	Tops.	Whole plants.	Total N content whole plants (mg.).	Relative values. Dry wt. whole plants.	N content.
21	7·17	41·76	48·93 (0·3)†	1,421 (27)†	100	100
12	7·18	43·67	50·85 (0·9)	1,378 (13)	97	97
5	6·43	42·30	48·73 (1·8)	1,493 (28)	99	105

* Data are per jar of three plants, with five jars per treatment. The plants received a total of 1,600 mg. nitrogen as $NaNO_3$ per jar during the experiment, and were harvested September 8.

† Figures in parentheses are standard errors of the means. To be significant, differences between means must exceed 3·67 gm. (whole plant dry weight) or 74 mg. (whole plant nitrogen) for $P = 0{\cdot}05$.

Data for the nodulated plants of the 1949 experiment are presented in Tables VII and VIII. The intervals before nodules appeared and fixation became obvious, and also details of pH control, were similar to those of the previous experiment. After 7 weeks' growth the plants of the air and 12 per cent. oxygen series, which had been growing more strongly than the 5 per cent. series, developed a chlorosis in the younger leaves, with some brown necrotic spots. The addition of extra iron as ferrous sulphate led in most cases to a rapid disappearance of these symptoms, which, it was concluded,

TABLE VII

*Data obtained at Harvest of Nodulated Plants of Experiment commenced April 30, 1949**

% oxygen in gas supplied.	No. of nodules.	Dry weights (g.).				Relative total.
		Nodules.	Roots.	Tops.	Total.	
21	1,371	2·00	4·49	17·26	23·75	
	1,555	1·90	4·25	15·95	22·10	
	1,747	1·95	4·03	17·12	23·10	
	1,565	1·94	4·39	16·69	23·02	
	(1,179)†	(1·63)	(3·36)	(14·46)	(19·45)	
Means	**1,560**	**1·95**	**4·29**	**16·76**	**23·00**	100
12	1,313	1·76	3·70	14·50	19·96	
	1,162	1·47	3·16	13·35	17·98	
	1,826	1·78	3·63	14·55	19·96	
	1,331	1·71	3·29	14·45	19·45	
	1,081	1·59	3·28	13·17	18·04	
Means	**1,343**	**1·66**	**3·41**	**14·00**	**19·08**	83
5	1,491	1·32	3·05	11·35	15·72	
	1,472	1·49	3·06	12·01	16·56	
	1,565	1·49	2·85	11·66	16·00	
	1,587	1·52	3·10	11·86	16·48	
	2,287	1·38	3·03	9·91	14·32	
Means	**1,680**	**1·44**	**3·02**	**11·36**	**15·82**	69

* Data are per jar of three plants, with five jars per treatment. These plants were harvested July 26.

† Figures in parentheses not included in means—see text.

TABLE VIII

Data for Mean Total Nitrogen of Plants of Table VII (mg. per jar of three plants)

% oxygen in gas supplied.	Nodules.	Tops and roots.	Whole plants.	Whole plants, relative basis.
21	87	354	441 (12)*	100
12	73	304	377 (11)	85
5	72	236	308 (12)	70

* Figures in parentheses are standard errors of the means. To be significant, differences between the means for whole plants must exceed 39 mg. ($P = 0{\cdot}05$).

had been due to a deficiency of iron in the culture solution; the plants in one jar of the air series failed to recover properly and the relevant data were not included in the calculation of mean values. Compared with the corresponding plants of the 1948 experiment it may be noted that (*a*) the 1949 nodulated plants made as a whole better growth, because of the earlier sowing date and more favourable weather, and (*b*) the effect of lowering the oxygen tension was generally similar but was less pronounced, particularly as regards the

5 per cent. level, this being no doubt partly due to the temporary set-back to the growth of the air and 12 per cent. series noted above. In addition to the effects on dry weight, the roots became shorter as oxygen tension fell, while the nodules tended to become aggregated to a greater extent on the upper part of the root system. Typical plants from this series are shown in Pl. VII, Fig. 3.

TABLE IX

*Data obtained at Harvest of Non-nodulated Plants receiving Ammonium-Nitrogen of experiment commenced April 30, 1949**

% oxygen in gas supplied.	Dry weights (g.).			Total N content whole plants (mg.).	Relative values.	
	Roots.	Tops.	Whole plants.		Dry wt. whole plants.	N content.
21	3·44	25·01	28·45	1,237		
	3·54	27·59	31·13	1,281		
	3·39	25·68	29·07	1,157		
	3·64	27·94	31·58	1,299		
	3·45	27·57	31·02	1,255		
Means	**3·49**	**26·76**	**30·25**	**1,246**	100	100
12	2·84	23·65	26·49	1,136		
	2·62	21·01	23·63	992		
	2·84	25·47	28·31	1,265		
	3·30	24·93	28·23	1,160		
	3·00	25·15	28·15	1,164		
Means	**2·92**	**24·02**	**26·96**	**1,143**	89	92
5	3·11	22·19	25·30	1,013		
	2·80	24·67	27·47	1,083		
	2·91	24·68	27·59	1,101		
	3·12	22·55	25·67	977		
	3·12	25·40	28·52	1,071		
Means	**3·01**	**23·90**	**26·91**	**1,049**	89	84

* Data are per jar of three plants, with five jars per treatment. The plants received a total of 3,600 mg. nitrogen as $(NH_4)_2 SO_4$ per jar during the experiment, and were harvested July 21.

Table IX shows the data for the plants of the 1949 experiment which received ammonium-nitrogen. The first addition of nitrogen was delayed until 32 days after transplanting,[1] and partly because of this there was not the same disparity in size between these and the nodulated plants that there was in 1948. In the 12-hour intervals between restoration to 6·5, pH fell appreciably, frequently to values in the region of 4·0, so that it is not claimed that the plants grew at a constant pH of 6·5. All the plants made very healthy growth with rich green leaves, individual leaflets attaining a length of 5 in., and to the eye no differences in growth were discernible between the three oxygen levels (Pl. I, Fig. 4). The harvest data show, however, that dry weights and nitrogen contents tended to decrease slightly at the lower oxygen levels, though compared with the nodulated plants the effects of reducing oxygen tension were slight. Root lengths were more affected, the mean lengths of the longest root on each plant being 87 (air), 78 (12 per cent. oxygen), and 50 cm. (5 per cent. oxygen) respectively.

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[1] The plants grew for a further 39 days after the first addition of combined nitrogen.

DISCUSSION

The technique employed in the present experiments consisted in exposing the root system to a controlled external oxygen tension, while the shoot system of the plant was exposed to the normal atmosphere of the greenhouse. This arrangement allows the shoot to receive more light and space for growth than is usually possible when the whole plant is subjected to adjusted oxygen tension, but at the same time the possibility has to be borne in mind of a downward diffusion of oxygen through the interior of the plant, under the influence of the difference in partial pressure of oxygen which exists when the root system is exposed to a reduced tension, and aided by the effect of photosynthesis. Such an event would make it impossible to exercise any close control over the oxygen supply in the tissues of the root merely by adjusting the oxygen content of the external solution. The oxygen data in Tables I–III do not, however, provide any indication of this internal movement of oxygen. For such a downward flow, if appreciable, would doubtless result in a tendency for oxygen to diffuse from the root system into the surrounding culture solution in the case of the cultures through which gas mixtures containing 12 or 5 per cent. oxygen were passing, so that the oxygen tension would at least be maintained at the equilibrium point, and might even rise above it if the diffusion of oxygen from the roots were too great for prompt removal by the gas stream. In actual fact, except in the earlier stages of the experiments, the oxygen content of the culture solution, both with nodulated and non-nodulated plants, was found to be consistently below the equilibrium point, despite vigorous gas-flow, leaving no doubt that the main movement of oxygen was into the root rather than out of it, and that oxygen tensions within the root did not exceed those in the external solution. Reference may also be made to a previous paper (Bond, 1950, Table VIII) where zero values for oxygen were recorded for samples of culture solution drawn from the base of non-aerated jars of soya-bean. Also relevant is the fact that the effects of lowered oxygen tension on growth reported in the present paper tend to exceed those obtained by other investigators with the plant as a whole exposed to adjusted oxygen tension (see later). The above considerations indicate that although evidence interpreted as demonstrating free movement of gases between shoot and root has been obtained for some plants (Glasstone, 1942), in the present experiments with soya-bean no extensive downward movement of oxygen occurred through the plants.

Reviewing briefly the observed effects of oxygen tension on growth, in two experiments with nodulated plants a progressive reduction in the dry weight attained by the whole plant and by its various organs was found on comparison of plants grown with their roots in culture solution in approximate equilibrium with oxygen/nitrogen mixtures containing 21, 12, and 5 per cent. oxygen respectively. A similar position held for fixation of nitrogen. Non-nodulated plants supplied with nitrate-nitrogen grew equally vigorously at all three oxygen levels, though this result is regarded with some reservation for reasons indicated. Plants supplied with ammonium-nitrogen showed, with 12 and 5 per cent. oxygen, slightly lower dry weights than the air series, but the extent of the reduction was considerably smaller than with nodulated plants. Thus taking the relative dry weight figures for the 5 per cent. oxygen series from the 1949 experiment, the reduction shown by the nodulated plants was nearly three times that shown by the plants receiving ammonium-nitrogen, the difference being still greater if the 1948 results with nodulated plants are taken into account.

It thus appears that when the oxygen supply is reduced from the air value it more quickly becomes limiting to the activities of nodulated root systems, dependent on the nodules for nitrogenous compounds, than to those of non-nodulated root systems supplied with combined nitrogen. If we assume that the roots alone of nodulated plants are similar in their oxygen relation to those of non-nodulated plants, then the conclusion is reached that it is the response of the nodules (or of the bacteria) that chiefly governs the reaction of the plant as a whole to oxygen tension.

If the position is analysed further, the ultimate effect of the reduced tension on the growth of the nodulated plants could *a priori* be envisaged as due to its action on one or more of the following stages: (*a*) the multiplication of the bacteria in the culture solution, (*b*) the penetration of the root-hairs by the bacteria, (*c*) the initiation of nodules, (*d*) the further development of the nodules, and (*e*) the processes of fixation and associated metabolism. The fact that the number of nodules was essentially unaffected by the oxygen treatments indicates that the first three stages are not specially sensitive to oxygen reduction, while (*d*), so far as it is not dependent on (*e*), is a matter of mitosis of legume cells, fission of bacteria, and structural differentiation, the first and last of which evidently proceeded without much hindrance under reduced oxygen supply in the roots of plants receiving combined nitrogen, while work cited by Wilson (1940) shows that the nodule bacteria in pure culture grow satisfactorily at low oxygen tension. Thus (*e*) remains as the aspect which is most likely to be involved in the explanation of the special effect of oxygen deprival on nodulated plants.

It does not necessarily follow from the above that nodules use more oxygen than root tissues. It might well be that structural differences render the access of oxygen to the interior of the nodule more difficult than with the root, so that, for the oxygen supply in the cells of each organ to be similar, a higher external tension would be necessary in the case of the nodule (Frazer, 1942; Smith, 1949). The observations of Bond (1941) and of Asprey and Bond (1941) by two methods on nodules and roots of soya-bean plants grown under similar conditions to those of the present experiment indicated, however, that the respiration of the nodule tissues, chiefly on the basis of carbon dioxide output and to a lesser extent on oxygen intake, was 2–3 times as intense per unit dry weight as that of root tissues, under conditions of ample air supply. The author's interpretation of the results reported in the present paper is that they are chiefly due to the greater rate of oxygen utilization by the soya-bean nodule demonstrated or implied by the previous work, and a consequent earlier limitation of nodule activities (including, perhaps indirectly, fixation of nitrogen) than of root activities when external oxygen is reduced.[1]

These results and conclusions may now be compared with those of Virtanen and von Hausen (1936) and of Wilson and Fred (1937). The first authors, after experiments in which the growth of nodulated pea plants in aerated and non-aerated culture solution and that of non-nodulated plants in non-aerated solution was observed, concluded that oxygen was of special importance for nodules. The present author's findings resemble those of Virtanen and von Hausen, but are based on more precise experimentation. Wilson and Fred, on the other hand, in experiments carried out on a considerable scale and at different seasons, found that the responses of nodulated and non-nodulated plants of clover to different conditions of oxygen supply were similar. With both there was (on the basis of the mean results for nitrogen content in the absence of hydrogen expressed in Fig. 1 of the paper) no reduction in growth until the partial pressure of oxygen was reduced to less than 0·05 atmosphere, and below this point growth was curtailed to about the same extent in each type of plant.

Wilson (1940), in seeking to reconcile the above results, has distinguished between the use of oxygen in the nodule directly in fixation, and indirectly in processes associated with fixation, examples of which may be bacterial or legume respiration.[2] The latter is, of course, the explanation favoured by the present author for his own observations. Wilson concluded that the work of Wilson and Fred provided no evidence of a direct utilization of oxygen in fixation, but it was agreed that oxygen has an indirect special importance for nodules and this was thought to explain the results of Virtanen and von Hausen. To the present author this does not seem to be a fully satisfactory explanation, for it is not clear why the indirect importance was not revealed in the experiments of Wilson and Fred. It is perhaps profitable to examine the techniques employed to see whether reasons for the discrepancies between the results of Wilson and Fred on the one hand and those of Virtanen and von Hausen and the present author on the other can be found there. The following points may be noted.

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[1] As pointed out previously (Bond, 1950), other workers, using material of different origin, have not found nodule respiration to be markedly different from that of roots when the organs are respiring in air. It should be noted, however, that Allison et al. (1940) found that in an atmosphere of oxygen nodule respiration became more than twice as great as that of roots, the respiration of the latter being unaffected by the increased oxygen. Their conclusion was that nodules are potentially capable of more rapid respiration than roots, though with the material and conditions that they employed nodule respiration was, in air, limited by a lack of oxygen in the nodule to a value which happened to be near that of roots. These results also would be in keeping with the finding that the nodule is more sensitive than the root to reduction of external oxygen supply.

[2] In a recent discussion of the mechanism of fixation Wilson and Burris (1947) visualize the possible use in the fixation process of energy-rich phosphate bonds. Presumably these would be of respiratory origin.

1. Virtanen and von Hausen and also the present author adjusted the oxygen supply only as regards the root system, and it is incumbent on those using this procedure to show that the oxygen available to the root is solely or chiefly that provided in the culture solution. Evidence in favour of the latter has already been advanced in respect of the present author's experiments.

2. Though no determinations of oxygen were reported in connexion with Virtanen and von Hausen's experiments, Smith (1949) has suggested that the oxygen tensions prevailing in the non-aerated jars of their experiments were below the lowest employed by Wilson and Fred (0·012 atmos.), and that this explains the divergent results. This assumes that had the last authors used still lower oxygen levels they would have found the growth of the nodulated plants to be handicapped more than that of non-nodulated plants. In view of Virtanen and von Hausen's use of unsealed containers with an air-space above the solution, it is probable that only in the later stages of the experiment could the suggested very low oxygen tensions have been attained.

3. In Wilson and Fred's experiments the plants were wholly enclosed in 2- or sometimes 10-litre bottles. With this technique the possibility of undue limitation of growth through lack of light and space has to be considered, since the full response of the plant to the more favourable oxygen levels might thereby be prevented. In those experiments for which details are given, the dry weight of nodulated red clover grown at a pO_2 of 0·2 atmosphere varied from 29 to 105 mg. per plant, indicating that sometimes the plants did not grow very strongly, though it is agreed that in some of these cases the considerably stronger growth made by the combined nitrogen plants shows that the available light and space permitted greater growth than the nodulated plants actually achieved.

4. Finally, it is possible that the identity of the leguminous plant may be important, and a complete explanation of the above discrepancies may not be possible until both techniques have been employed with a given plant.

A grant from the Agricultural Research Council has facilitated this investigation, in which technical assistance has been given by Dr. M. P. McGonagle and Messrs. T. P. Ferguson, J. C. Leitch, and D. Wilkie. Mr. W. Anderson assisted with the photographs.

LITERATURE CITED

ALLISON, F. E., LUDWIG, C. A., MINOR, F. W., and HOOVER, S. R., 1940: Biochemical Nitrogen Fixation Studies. II. Comparative Respiration of Nodules and Roots, including Non-legume Roots. Bot. Gaz., ci. 534–49.

ASPREY, G. F., and BOND, G., 1941: Respiration of Leguminous Root Nodules. Nature, cxlvii. 675.

BOND, G., 1941: Symbiosis of Leguminous Plants and Nodule Bacteria. I. Observations on Respiration and on the Extent of Utilization of Host Carbohydrates by the Nodule Bacteria. Ann. Bot., N.S., v. 313–37.

—— 1950: Symbiosis of Leguminous Plants and Nodule Bacteria. III. Observations on the Growth of Soya Bean in Water Culture. Ibid., N.S., xiv. 245–61.

FRAZER, H. L., 1942: The Occurrence of Endodermis in Leguminous Root Nodules and Its Effect upon Nodule Function. Proc. Roy. Soc. Edin. B, lxi. 328–43.

GILBERT, S. G., and SHIVE, J. W., 1942: The Significance of Oxygen in Nutrient Substrates for Plants. I. The Oxygen Requirement. Soil Sci., liii. 143–52.

—— —— 1945: The Importance of Oxygen in the Nutrient Substrate for Plants—Relation of the Nitrate Ion to Respiration. Ibid., lix. 453–60.

GLASSTONE, V. F. C.-, 1942: The Passage of Air through Plants and its Relation to Measurement of Respiration and Assimilation. Amer. Journ. Bot., xxix. 156–9.

SMITH, J. D., 1949: Haemoglobin and the Oxygen Uptake of Leguminous Root Nodules. Biochem. Journ., xliv. 591–8.

VIRTANEN, A. I., and VON HAUSEN, S., 1936: Investigations on the Root Nodule Bacteria of Leguminous Plants. XVII. Continued Investigations on the Effect of Air Content of the Medium on the Development and Function of the Nodule. Journ. Agr. Sci., xxvi. 281–7.

WILSON, P. W., 1940: The Biochemistry of Symbiotic Nitrogen Fixation. Madison. 302 pp.

—— and BURRIS, R. H., 1947: The Mechanism of Biological Nitrogen Fixation. Bact. Rev., xi. 41–73.

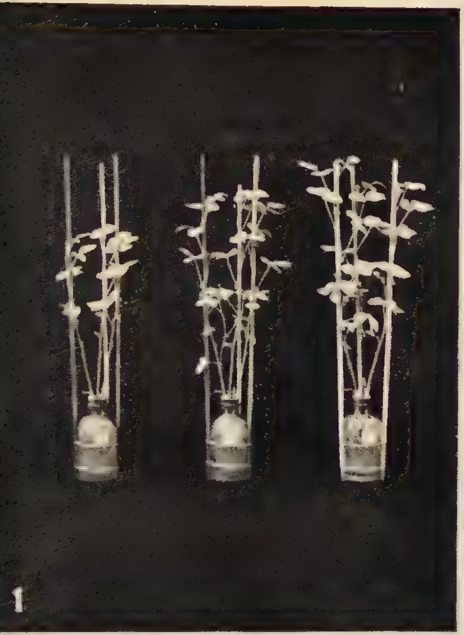
—— and FRED, E. B., 1937: Mechanism of Symbiotic Nitrogen Fixation. II. The pO_2 Function. Proc. Nat. Acad. Sci., xxiii. 503–8.

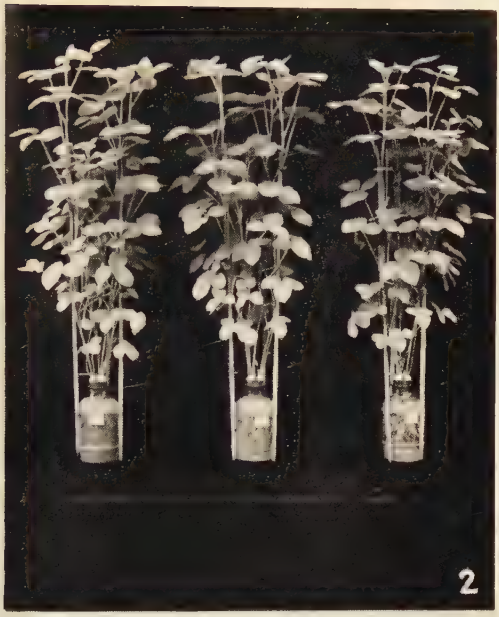
EXPLANATION OF PLATE VII

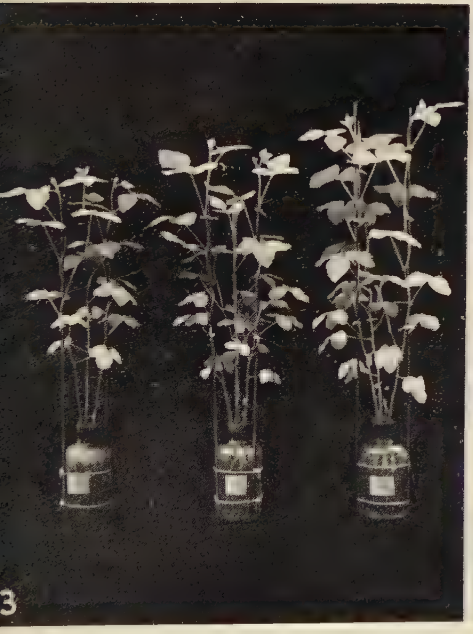
Illustrating G. Bond's paper on 'Symbiosis of Leguminous Plants and Nodule Bacteria. IV. The Importance of the Oxygen Factor in Nodule Formation and Function.'

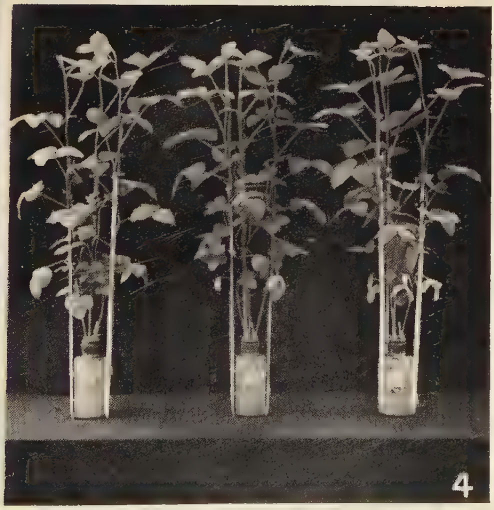
In each photograph the cultures of soya-bean, reading from left to right, had received oxygen/nitrogen mixtures containing respectively 5, 12, and 21 per cent. oxygen. The photographs were taken shortly before harvest, and as a guide to scale it may be noted that the actual height of the culture jars was 25 cm.

Fig. 1. Nodulated plants of the 1948 experiment.
Fig. 2. Non-nodulated plants receiving nitrate-nitrogen, 1948 experiment.
Fig. 3. Nodulated plants of the 1949 experiment.
Fig. 4. Non-nodulated plants receiving ammonium-nitrogen, 1949 experiment.









G. BOND

Studies on the Germination of the Seeds of *Striga hermonthica*

III. On the Nature of Pretreatment and after-ripening

BY

K. B. VALLANCE

(*The Hartley Botanical Laboratories, the University of Liverpool*)

With nine Figures in the Text

ABSTRACT

An attempt has been made to elucidate some of the changes which take place in seeds of *Striga hermonthica* (immature, ripe and overripe), (*a*) during moisture-treatment at three temperatures (15° C., 22° C., and 32° C.), and (*b*) during after-ripening in dry storage.

Observations on the drift of respiration of immature seed throughout pretreatment at 15° C. show that an inverse relationship exists between germination and respirational activity. This suggests that germination depends on the accumulation of some metabolite during pretreatment. It would seem that this metabolite may also be used as a substrate for respiration during pretreatment.

After-ripening of the immature seeds has been shown to change the nature of the seeds in some way so that their peak respiration on exposure to moisture is at first progressively reduced. This reduction has been ascribed to the presence of some barrier capable of restricting gaseous diffusion.

The data presented, together with those of the previous paper of this series, suggest that the specific metabolite required for germination might be proteinaceous. Certain other data appear to rule out the suggestion that germination capacity is conditioned by the sugar content of the seeds. Reasons are given which support the view that one of the main effects of the stimulating solution on the seed is to increase the permeability of the seeds to gaseous diffusion.

INTRODUCTION

IT has been shown by Andrews (1945) that optimum germination of the seeds of *Striga hermonthica* cannot be induced unless they are exposed to a suitable stimulating solution. This observation was confirmed by the present author (Vallance, 1950 *a*), who also showed that the seeds must first be subjected to a period of moisture-treatment at a temperature between 12° and 22° C., if their optimum germination in the presence of the stimulating solution is to be realized. With regard to the need for moisture-treatment, the germination of the seeds of *Striga hermonthica* is similar to that of *Orobanche speciosa* (Chabrolin, 1938) and *Striga lutea* (Brown and Edwards, 1944). Furthermore, the work described in both the earlier papers of this series (Vallance, 1950 *a* and 1950 *b*) suggested that this moisture-treatment, or pretreatment as it has been termed, is not necessary merely to increase the

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permeability of the seeds to moisture and to gaseous exchange, but to permit the attainment of certain essential stages in the germination process. These latter conclusions for *Striga hermonthica* are in agreement with those of Brown and Edwards (1946) in the case of *Striga lutea.* The nature of the changes which take place within the seeds during pretreatment was not established in the previous work, but differences in the respiration of pretreated and non-pretreated seeds, after their exposure to a natural stimulating solution, suggested that part of the germination process might entail the accumulation of some metabolite during the first phase of pretreatment. This metabolite, in the case of seed which is not overripe, would seem to disappear during prolonged pretreatment.

In view of the above considerations it was thought that it would be a matter of some interest to elucidate the changes which must take place during pretreatment. Clearly, however, a full investigation of the nature of pretreatment would require extensive chemical analysis at intervals throughout pretreatment, together with parallel observations on water uptake and respiration. Such an investigation would require a large quantity of freely germinable seed, which unfortunately it has not yet been possible to obtain. Nevertheless it was considered that, using the material available, certain investigations could be carried out which would serve to clarify some aspects of the pretreatment process, the after-ripening process, and the effect of the stimulant on the seeds. Thus studies were undertaken of the drift of respiration during the pretreatment of seeds of four ages at various temperatures, and of the effect of the stimulating solution on the respiration of overripe seeds. The changes in the sugar content and crude fat content during the pretreatment of a sample of immature seed were also investigated. These studies form the subject-matter of the present paper.

As previously, the author would like to acknowledge his indebtedness to Dr. F. W. Andrews (of the Gezira Research Farm, the Anglo-Egyptian Sudan) and to the Empire Cotton Growing Corporation, for supplying seed of *Striga hermonthica.* The author would like also to thank Dr. W. O. James for helpful suggestions in relation to some of the respiratory data presented.

EXPERIMENTAL METHODS

The methods of preparing the stimulating solution (using seedlings of Sudan grass), of moisture-treating the *Striga* seeds, and of inducing the germination of the *Striga* seeds were the same as those described in an earlier paper (Vallance, 1950*a*).

Generally, for each experiment on the respiration of the seeds during pretreatment, 0·5 g. of seed were placed in each of fourteen sintered crucibles. These seeds were then exposed to moisture at an appropriate temperature. At intervals throughout the pretreatment periods one of the crucibles was selected at random, and the seeds inside were washed thoroughly and divided into six approximately equal portions. Each of these portions was then introduced into a respirometer flask. When the quantity of seed available permitted, samples of seed from a single pretreatment vessel were introduced into each of a pair of respirometers, and hence in these experiments each of the three pairs of determinations of oxygen uptake and carbon dioxide evolution were independent. The respiration of the seeds was always measured at a temperature of 32° C. Dixon's modification of the Barcroft apparatus was used in some experiments, whilst standard Barcroft respirometers were used in others, but in any one experiment six respirometers of the same type were used together. The respirometer flasks used with each respirometer were matched for volume as closely as possible. In consequence, it was considered that when the influence of the stimulating solution on seed respiration was being measured the possible effects of this solution on the composition of the gases inside the flasks could be neglected. As a further precaution, in experiments with the stimulating solution care was taken to introduce approximately 70 mg. (dry weight) of seed each time into the respirometer flasks.

At the end of each determination of seed respiration the contents of each right-hand side respirometer flask were transferred to a small sintered crucible. The water was then filtered off from these crucibles, and after this the crucibles and their contents were dried for 16 hours at a temperature of 80° C. The seeds inside each were then weighed.

Results are expressed as μl. of gas at N.T.P. per mg. dry weight per hour, and each point plotted in the figures illustrating respiration is the mean value of three determinations. Data were analysed for variance whenever this was thought to be necessary.

In certain experiments parallel estimations of respiration and of the sugar content were carried out. Sugar content was estimated by the method of Weinmann (1944, 1947). Sufficient material was not available to enable the precise nature of the seed sugars to be determined. Results are therefore expressed as mg. per gramme dry weight of hexose before hydrolysis and as mg. of increase in hexose content after acid hydrolysis.

Experimental Results

Altogether four batches of seed were available. The germination of the seeds of three of these batches did not exceed 0·1 per cent. in the absence of the stimulating solution, and thus these seeds appeared to be immature. Forty per cent. of the seeds of the fourth batch were induced to germinate in the absence of the stimulating solution. It therefore seemed reasonable to regard the seeds of the latter batch as fully ripe. The respiratory data obtained from experiments in which ripe and overripe seeds were employed showed certain differences from that concerning immature seeds, and hence the respective sets of data are considered in separate sections.

In all experiments it was thought that the respiration of the seeds during the second hour inside the respirometers could be taken as an index of the respiration of the seeds immediately prior to their being placed in the respirometer flasks.

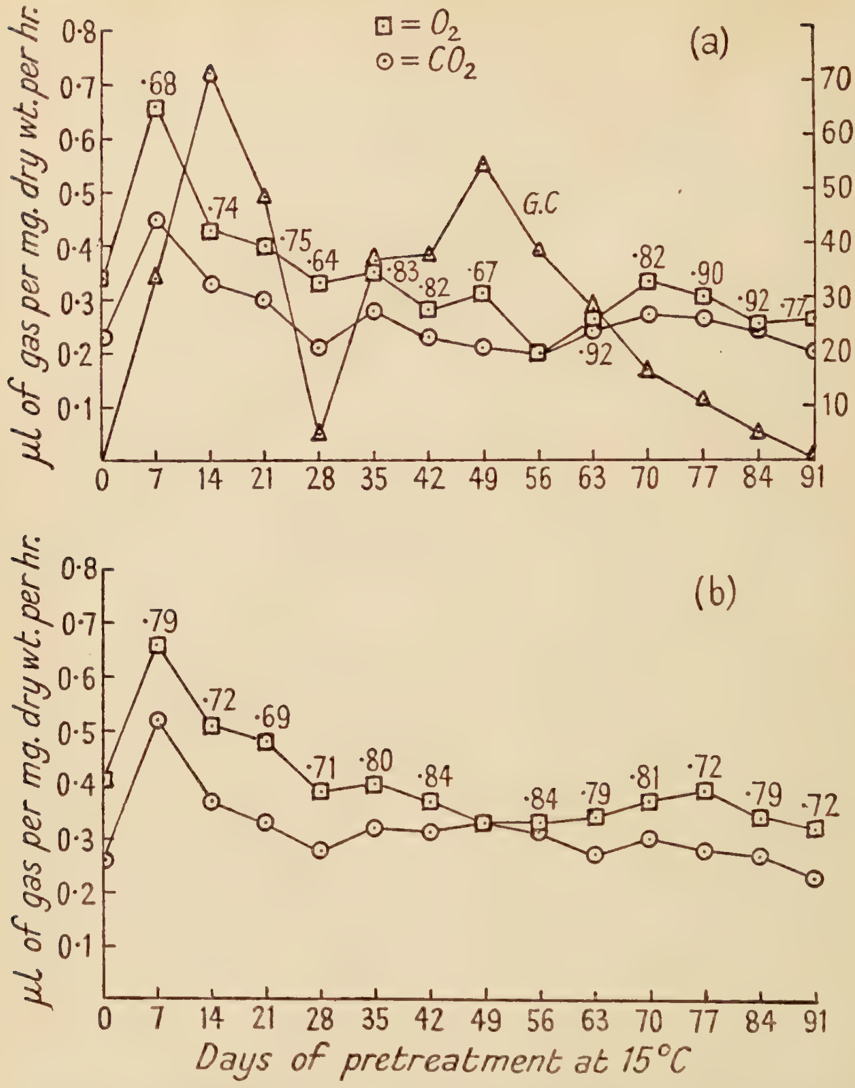

FIG. 1. *a.* The relationship between length of pretreatment at 15° C., respiration, and germination capacity in the presence of the stimulating solution (G.C.). 1945 seed. Pretreatment initiated September 1947. *b.* The effect of length of pretreatment at 15° C. on the gaseous exchanges of 1945 seed during the 7th hour inside the respirometer flasks. (Standard error of each plotted value of respiration in *a* and *b* = ±0·022.)

The respiration of immature seeds

Fig. 1*a* illustrates the drift of respiration obtained by pretreating 1945 seeds at 15° C. for varying periods up to 91 days and sampling once per week. Pretreatment was initiated in September 1947. Fig. 1 *b* shows the respiration of the same 1945 seed as that used to obtain the data of Fig. 1 *a* during the

7th hour inside the respirometers. The plotted values of the respiratory data shown in Fig. 1 *a* and Fig. 1 *b* are the mean values of three independent pairs of determinations. The figures printed near each of the oxygen values in all graphs represent the R.Q.s at the time of each observation.

It will be immediately apparent from Fig. 1 *a* that there was no correlation between respiration and the germination capacity of the seed at any stage of pretreatment. Hence the attainment of the maximum germination capacity of the seed at this stage of ripeness cannot be related to its metabolic activity, as measured by the rate of respiration. Fig. 1 *a* also shows that the maximum germination capacity of the seed, during the first germination cycle, was not attained until oxygen absorption and carbon dioxide evolution had fallen from 0·66 to 0·45 μl. per hour to 0·43 and 0·32 μl. respectively. In consequence, that section of the data obtained between 0 and 14 days might suggest that the initial attainment by the seeds of the capacity to germinate depended in part on the accumulation of some metabolite which could also be used as a respiratory substrate. However, unless it can be accepted that the rate of mobilization of this metabolite varies during pretreatment, the hypothesis will not account for the fall, rise, and final drop in the germination capacity observed between 14 and 91 days. Nevertheless, earlier work (Vallance, 1950 *b*) on the effect of the stimulating solution on seed respiration would seem to support the view that the capacity of the seeds to germinate may depend in part on the availability of some metabolite. Certainly, when seeds were pretreated at 22° C. for 0, 6, and 33 days respectively and were then exposed to the stimulating solution, the nature of the respiration of the seeds pretreated for 6 days was different from that at the other stages of pretreatment. At the 6th day, when up to 50 per cent. of the seeds were induced to germinate, it seemed that a protein might be acting as the respiratory substrate, whereas at the other times it seemed that the substrate for respiration was largely carbohydrate. After 0 and 33 days of pretreatment little germination took place.

With regard to the data of Fig. 1 *a* it would seem that if germination depended on the availability of some specific metabolite, then its accumulation in the later stages of pretreatment would be due to the combined effects of two factors. These would be the rate of mobilization of the metabolite and the rate of respiration. Hence one prerequisite for the attainment of the capacity to germinate would be a low level of respiration, which might be brought about by some such phenomenon as carbon dioxide narcosis. The possibility that carbon dioxide narcosis might in fact have been responsible for the low levels of respiration shown in Fig. 1 *a* between the 14th and 91st days was suggested by the data of Fig. 1 *b* in conjunction with the data of Figs. 1 *b* and 2 of the previous paper (Vallance, 1950 *b*). Figs. 1 *b* and 2 of the previous paper show that temperature has a marked effect on the respiration of seeds during the first 12 hours of moisture-treatment. From Fig. 1 *b* of the present paper, which shows the respiration of seeds during the 7th hour inside the respirometers, it will be seen that the increases in the respiratory rate compared with Fig. 1 *a* were not as great as might have been expected had the Q_{10} principle alone been operative. On the other hand, if the seeds had been subjected to internal carbon dioxide narcosis, then the increase in temperature

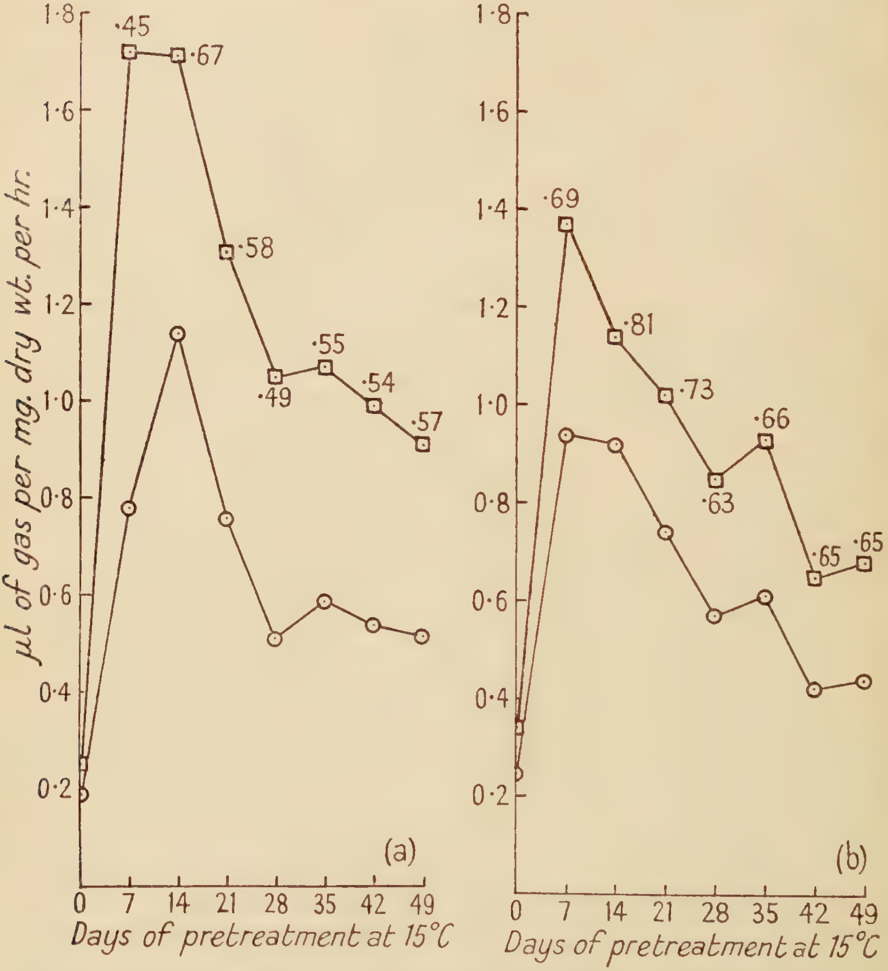

FIG. 2. *a* and *b*. The effect of length of pretreatment at 15° C. on the gaseous exchanges of 1949 seed during the 2nd (Fig. 2 *a*) and 7th hours (Fig. 2 *b*) respectively at 32° C. Pretreatment initiated November 1949. (Standard error of each plotted mean = ±0·066.)

from 15° to 32° C. might, as shown by Kidd (1914, 1915) and Kidd and West (1917) in the case of *Sinapis alba*, tend to minimize its effects. The observed increases in the rate of respiration from the 2nd to the 7th hours would seem to be in accord with this hypothesis.

The theory postulated above suggests that if the seeds of *Striga hermonthica* respire at a comparatively high level during pretreatment, then their germination capacity will be small. Experiments using seed of four ages supported this theory in general, and the negative correlation between high respiration and absence of germination was most apparent when the respiration of the least mature seeds was measured during pretreatment. A typical example of this is shown in Fig. 2 *a*, in which all values are the means of three independent determinations. This figure illustrates the drift of respiration of 1949 seeds during their pretreatment at 15° C. in the months of December 1949 and January 1950. These 1949 seeds could not be induced to germinate during the experiment. It will be seen from Fig. 2 *a* that, over a comparable period, the rate of gaseous exchange of the 1949 seeds was much greater than that of 1945 seeds shown in Fig. 1 *a*. As in Fig. 1 *a*, however, Fig. 2 *a* shows an initial rise in the intensity of respiration followed by a later fall.

It will also be noticed that the R.Q.s shown in Fig. 2 *a* are different from those observed at the comparable stages in Fig. 1 *a*. The R.Q.s of Fig. 2 *a* suggest a protein respiration, whereas those of Fig. 1 *a* suggest either a fat plus carbohydrate respiration or, alternatively, a protein-carbohydrate respiration.

Now Fig. 2 *b* shows that the rate of oxygen intake during the 7th hour by the 1949 seeds was generally significantly lower than that during the 2nd hour, and hence it seems unlikely that carbon dioxide narcosis could have been influencing respiration in this case. Moreover, differences in the R.Q.s in Figs. 1 *a*, 1 *b*, 2 *a*, and 2 *b* would suggest that carbohydrate was involved in the respiration of the germinable 1945 seed. It seemed reasonable, therefore, in spite of the data on the effect of the stimulating solution on seed respiration, to consider the possibility that the 'specific metabolite' required for germination might be a sugar. Apart from the data on the R.Q.s this possibility required examination, as Saunders (1933) has reported that at the times when the seeds of *Striga lutea* were germinating they contained large quantities of sugar. When this sugar hypothesis was first considered, data were available which showed the changes in sugar content, water content, respiration, and germination capacity during the pretreatment of samples of 1948 seed at a temperature of 20° C. (A pretreatment temperature of 20° C. was employed in these experiments in order to avoid the complications sometimes associated with pretreatment at 22° C., as discussed on p. 113.) Examination of these data showed that the 'specific metabolite' could not be sugar alone, although sugar seemed always to be present at times when the seeds were able to germinate. The complete data of one experiment are shown in Fig. 3.

It will be seen from Fig. 3 that the maximum germination capacity of the sample of 1948 seed, at the time of the experiment, was 18 per cent. As in Fig. 1 *a*, the maximum germination capacity of the seed was not attained until the carbon dioxide output had fallen to a significantly lower value than the initial peak value. Comparison of the sugar curves of Fig. 3 with those of respiration and germination shows that from 0 to 3 days, within the experimental limits, the rate of gaseous exchange increased from 0·24 μl. of oxygen and 0·22 μl. of carbon dioxide to 0·75 μl. and 0·58 μl. respectively; the sugar content diminished to about a sixth of its value at 0 days, and germination was in the neighbourhood of 1 per cent. From 7 to 14 days, when the germination capacity was increasing, the carbon dioxide output fell to 0·38 μl. and the sugar content increased up to the 10th day. However, from 14 to 37 days the non-reducing sugar content increased to 8·5 mg., but this increase was not accompanied by a rise in germination capacity. Furthermore, estimation of the water content of the seeds during pretreatment gave no indication that the germination capacity might be due to the presence of some other osmotically active substance. This may also be seen from Fig. 3, which suggests

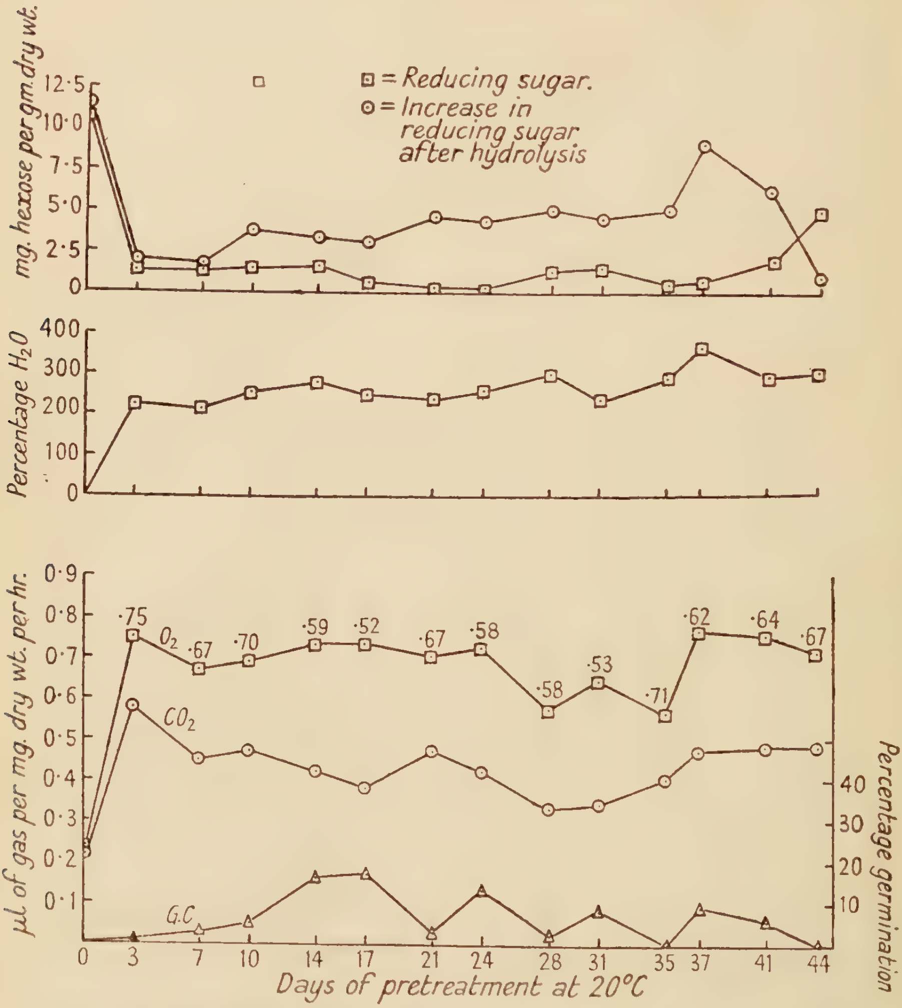

FIG. 3. The effect of length of pretreatment at 20° C. on the respiration, water content, sugar content, and germination capacity (G.C.) of 1948 seed. Pretreatment initiated September 1948. (Standard error of each plotted respiration value = ±0·036.)

that from 7 days onwards the increase or decrease in the water content was largely the result of variation in the sugar content of the seeds.

The amount of 1948 seed available for pretreatment at the time of the experiments illustrated in Fig. 3 permitted a few determinations to be made of the crude fat content at certain stages of pretreatment. It was found that the initial weight of crude fat was 1·34 per cent. of the dry weight (mean of two determinations). After 37 and 44 days of pretreatment it was 4·53 and 4·30 per cent. respectively. In view of this and other unpublished data it seemed unlikely that the substrate for respiration during the pretreatment of the 1948 seeds was a fat. It seemed more probable that the respiratory substrate was largely protein; and furthermore, in view of the small amount of crude fat in the dry seeds, it is thought that protein always forms part of the substrate for respiration during pretreatment.

For purposes of comparison with the respiratory and germination data of Fig. 3 the relevant data of an experiment on 1945 seeds are included. This sample of 1945 seed was pretreated in July 1947 at a temperature of 22° C. The data of this experiment are shown in Fig. 4. From this figure it may be seen that the curve of germination capacity was cyclic and that the maximum germination capacity was 50 per cent. Interpolation between 3 and 6 days in the curve showing the drift of respiration indicates that, as in previous data, the initial peak germination capacity was not reached until the rate of carbon dioxide output had begun to diminish. Most significantly, it will be seen that the peak values of both oxygen input and carbon dioxide output (obtained by interpolation) were lower than those shown in Fig. 3. In addition the R.Q.s calculated for each period over which respiration was measured were, with one exception, higher after the 3rd day than those shown in Fig. 3 and showed similar fluctuations to those given in Fig. 1 *a*.

At this stage it seems convenient to draw attention to some of the implications of the after-ripening process. It would seem that, during the first phase of after-ripening, certain changes take place gradually inside the seeds. As a result, the maximum rate of respiration which may be attained, during pretreatment at a temperature of between 15° and 22° C., is at first progressively restricted as the seed ages. This restriction in the maximum rate of respiration is intimately bound up with the germination process, for until this peak respiration falls below a critical value germination cannot take place.

The data given previously relate to respiration during the pretreatment of *Striga* seeds at temperatures of between 15° and 22° C. These data have some features in common, but, rather strangely, the same type of respiratory drift was not obtained when seeds were pretreated at higher temperatures.

Fig. 5 shows the drift of respiration of successive lots of 1945 seeds during pretreatment at 32° C. in the months of May and June 1947. Similar data were obtained from the replicate experiment. The data shown in Fig. 5 for the period 0 to 9 days were in accordance with expectation, in that the rate of respiration first increased, though rather more rapidly than in the other experiments in which this seed was used, and then diminished. From 1 to 9 days, when the rate of gaseous exchange fell progressively, the germination capacity increased. Again as was expected, in view of the comparatively high level of respiration at the end of the first day, the peak germination capacity was only of the order of 15 per cent. The results obtained during the

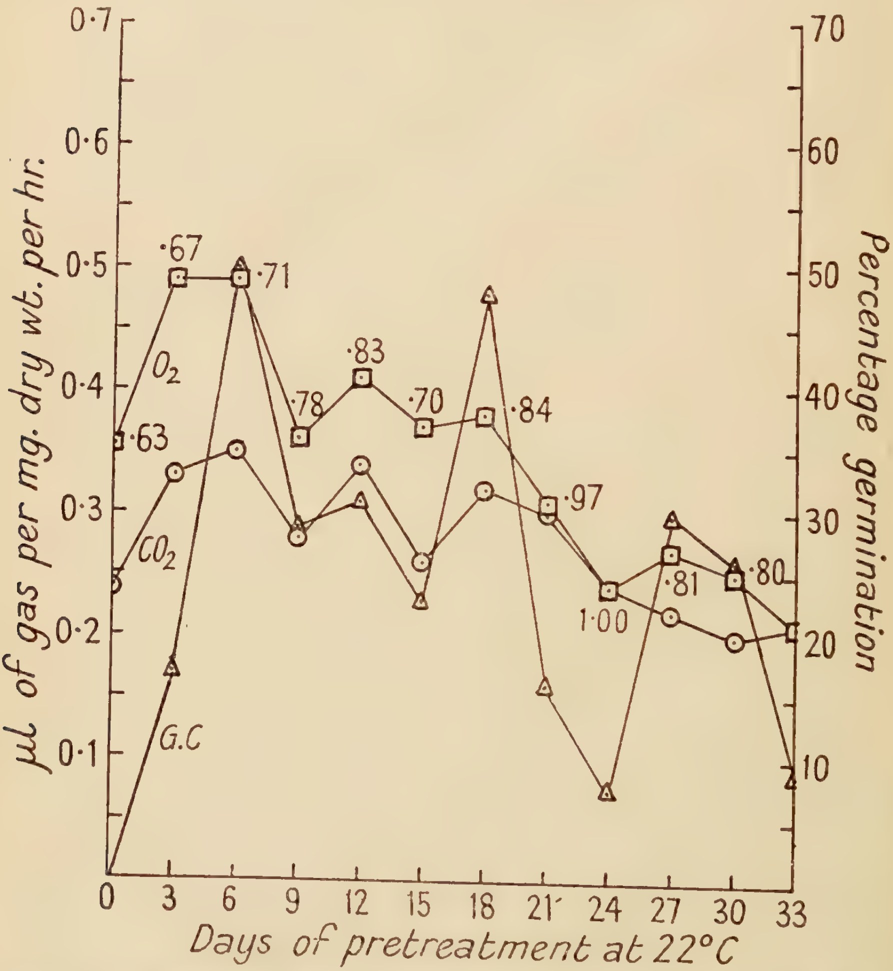

FIG. 4. The relationship between length of pretreatment at 22° C., respiration, and the germination capacity (G.C.) of 1945 seed. Pretreatment initiated July 1947. (Standard error of each plotted value of respiration = ±0·030.)

remainder of the experimental period show certain features which cannot yet be explained. It will be seen that from 9 to 33 days the intensity of the respiration increased, diminished, and then rose and fell once more before settling down to a comparatively low level. The reasons for the cyclic rise and fall in the drift of respiration shown in Fig. 5 are still obscure, but even so the data presented serve to emphasize one important fact. This is, that

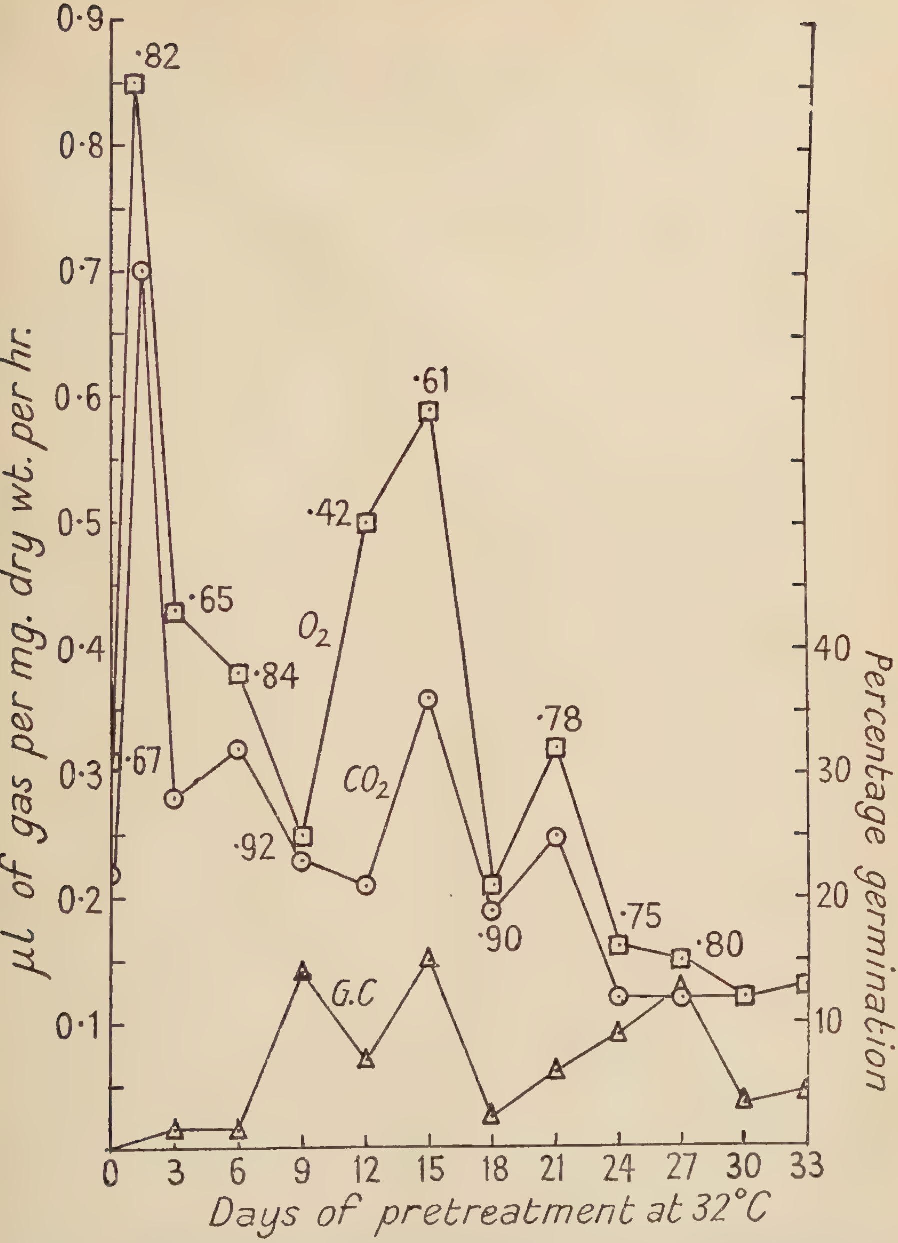

FIG. 5. The effect of length of pretreatment at 32° C. on gaseous exchange and germination capacity (G.C.) of 1945 seeds. Pretreatment initiated June 1947. (Standard error of each value of respiration = ±0·030.)

until the seed is fully mature its germination capacity is conditioned by its rate of respiration during the first few days of pretreatment.

Fig. 6 shows the drifts of respiration and germination capacity during the pretreatment of 1945 seed at a supposed temperature of 22° C. during the months of June and July 1947. It would seem that the major differences between the data of Figs. 4 and 6 were due to a slight increase in temperature during the first 2 days of pretreatment in the latter case. Earlier work (Vallance, 1950 *a*) on the effect of temperature on germination confirms this view; this is also supported by the fact that the drift of respiration shown in Fig. 6 has more features in common with that of Fig. 5 than with previous data.

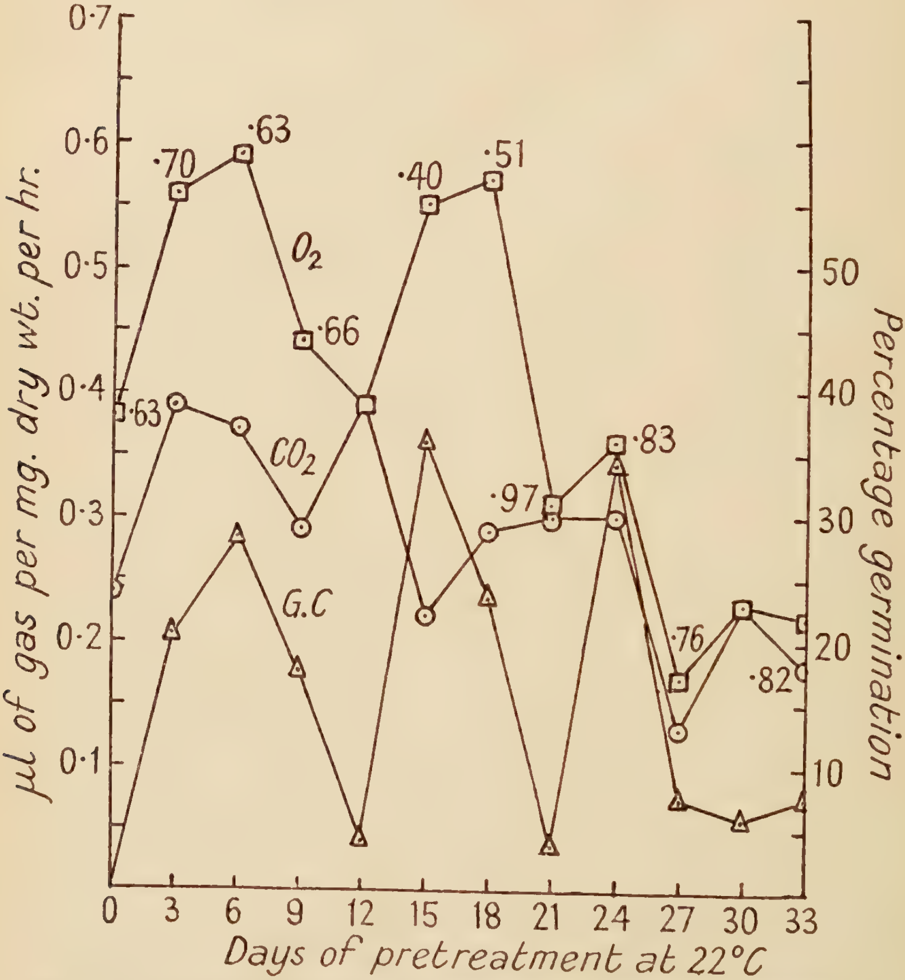

FIG. 6. The relationship between length of pretreatment, apparently at 22° C., respiration, and the germination capacity (G.C.) of 1945 seed. Pretreatment initiated June 1947. (Standard error of respiratory data = ±0·036.)

No satisfactory explanation can as yet be offered to account for the cyclic changes in the drift of respiration shown in Fig. 6. Possibly they may be due to changes in the permeability of the outer layers of the seed to gaseous diffusion during pretreatment, but this has not been established. Nevertheless, interpolation between 3 and 6 days in the drifts of respiration and germination capacity indicates that the initial peak value of the germination capacity was not attained until the rate of carbon dioxide output had begun to fall. Furthermore, it will be seen that when the R.Q.s approached unity the germination capacity was at its lowest value. This latter phenomenon was also noticed in the data shown in Fig. 4.

It would seem that the final fall in the drift of respiration to the low level at the 33rd day which is shown in Figs. 4 and 6 must be due to some kind of narcosis and not to the death of the majority of the seeds. This seems clear from previous work in which it was shown that a large proportion of a sample of immature seed could still be induced to germinate even after 51 days of pretreatment at 22° C. (In these experiments the pretreated seeds were dried over sulphuric acid for 48 hours, and were then pretreated once more at 22° C. and exposed to the stimulating solution in accordance with the standard method.) The apparent narcosis might be an internal carbon dioxide narcosis, for in some respects the treatment employed to overcome the secondary dormancy was similar to that used by Kidd (1914, 1915) in the case of the seeds of *Sinapis alba*. This worker dried out seeds of *Sinapis alba* after exposing them to moisture and to carbon dioxide for long periods and found that they would germinate on being moistened with water. Now if the immature seeds are subject to internal carbon dioxide narcosis after prolonged periods of pretreatment at 22° C., then this narcosis may also inhibit the mobilization of the so-called 'specific metabolite'. This was shown when the effect of the stimulating solution on seed respiration was measured after the seeds had been exposed to moisture for 33 days at 22° C. The nature of the respiration observed in these experiments suggested that the respiratory substrate was mainly carbohydrate, whereas at a time when the seeds could germinate freely the respiration seemed to be either partly or entirely protein respiration.

The drift of respiration of ripe and overripe seeds during pretreatment at a temperature of 22° C.

In May 1947 the only freely germinable seed of *Striga hermonthica* available was harvested in 1941. In view of the known high germination capacity of this seed, it was initially considered that a study of the drift of respiration of this seed throughout pretreatment at 22° C. would give rather more information on the nature of pretreatment than would a comparable study using 1945 seed. In actual fact the experimental results obtained differed markedly from those of all other experiments, but nevertheless replicated experiments gave results which were not significantly different in any respect. Hence it is thought that the differences shown between the data obtained from the

1941 seed and that given earlier are due to the effects of after-ripening on the seeds and not to the experimental conditions.

Figs. 7 *a* and 7 *b* illustrate the results obtained during the pretreatment at 22° C. of 1941 seeds. The data of the replicate experiment are not significantly different and consequently are not given. Fig. 7 *a* shows the drift of respiration (2nd hour inside the respirometers), the germination capacity in the presence of the stimulating solution, and the capacity for germination at 32° C. independently of the stimulant. Fig. 7 *b* illustrates the respiration during the 5th hour inside the respirometers after the amounts of pretreatment shown.

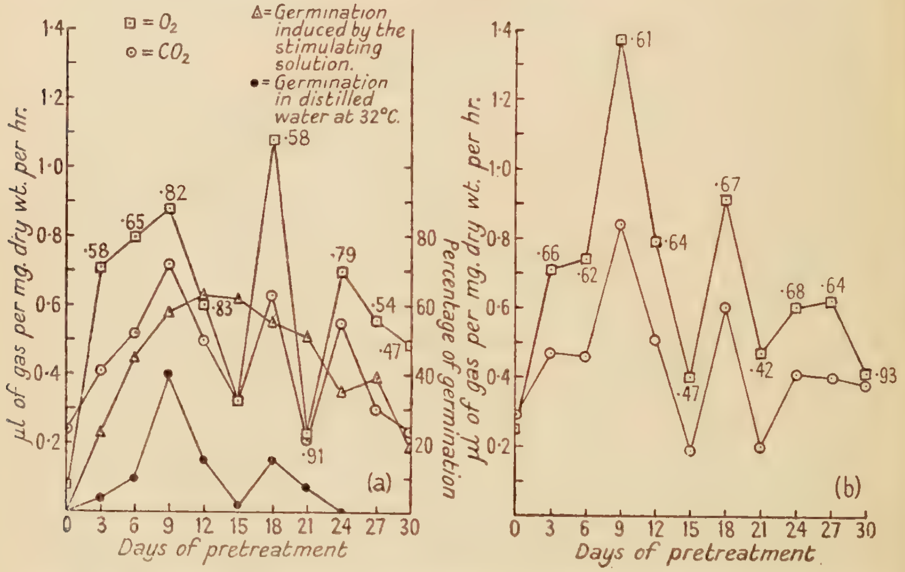

FIG. 7. *a*. The effect of length of pretreatment on the respiration and germination of 1941 seeds. Pretreatment initiated April 1947. *b*. The effect of length of pretreatment at 22° C. on the gaseous exchanges of 1941 seed during the 5th hour inside the respirometer flasks. (Standard error of respiratory data of Figs. 7 *a* and 7 *b* = ±0·060. Standard error of germination data = ±2·3.)

Now in contrast to the data given earlier, the data of Fig. 7 *a* show that when the seeds are fully ripe their germination capacity is governed, at least in the early stages of pretreatment, by their metabolic activity. This may be seen from the results for the 0- to 9-day period. In this period the input of oxygen and output of carbon dioxide rose from 0·08 μl. and 0·22 μl. to 0·88 μl. and 0·72 μl. respectively, and the R.Q. increased from 0·58 at the 3rd day to 0·82 at the 9th. During the same 9 days the germination capacity in the presence of the stimulating solution rose from 0 to 58·0±2·3 per cent. and the independent germination increased from 0 to 40·0±2·3 per cent. It will also be seen from Fig. 7 *b* that at the 9th day, when the capacity for independent germination reached 40·0 per cent., the respiration increased markedly between 2 and 5 hours. If this is taken as evidence that the seeds themselves had manufactured the active principle of the stimulating solution at this stage (in view of the known effect of the stimulant on respiration), then it is curious that the respiration did not increase significantly between 2 and 5 hours at other times when the independent respiration was 10 per cent. or higher. It will be noticed, for example, that after 18 days of pretreatment the amount of independent germination was 15 per cent., yet oxygen consumption fell from 1·07 μl. to 0·90 μl. in the relevant period.

From Fig. 7 *a* it may also be seen that from 9 to 18 days the variation in the germination capacity, in the presence of the stimulating solution, was not significant and yet the rate and nature of the gaseous exchanges varied markedly and significantly from one observation to the next. The incompleteness of the available data makes interpretation of these phenomena difficult, but inspection of the data of Fig. 10 of the first paper of this series (Vallance, 1950 *a*), in conjunction with that of Fig. 7 *a* of the present paper, suggests certain possibilities which may be of some interest. It would seem, for example, that even though the germination capacity of the 1941 seed did not vary significantly from 9 to 18 days, the effect on germination of diluting the stimulating solution would have been most marked at the 15th day. Now if it is assumed that the stimulating solution helps to promote one or more specific reactions inside the seeds, and that for germination to take place these reactions must proceed at a certain rate, then the reaction rates will depend on two factors, neglecting temperature. These will be the concentration of the active principle of the stimulating solution and the concentration of the appropriate substrate or substrates. Brown and Edwards (1946) suggest, in the case of *Striga lutea*, that the seeds may manufacture the stimulant themselves, with varying efficiency, during the first part of pretreatment, and that the dilution effects are due to the seed requiring a certain threshold amount of the stimulant before germination will take place. If this view is correct, then when germination is induced by the stimulating solution, part of the requisite amout of stimulant is provided by the seeds and the remainder diffuses in from outside. On the other hand, with a fixed external concentration of the stimulant, variation in the rate of the specific reaction or reactions could be brought about by varying the concentration of the substrate, or specific metabolite as it has been termed earlier. With regard to the seeds of *Striga hermonthica*, it is thought that the dilution effects are best explained on the latter assumption. On this assumption the concentration of the specific metabolite during the 9- to 18-day period of Fig. 7 *a* would have varied from day to day, and would have been lowest at the 15th day when the R.Q. was unity. Hence the minimum concentration of the active principle of the stimulating solution required to produce optimum germination would not have been the same at all times and would have been greatest at the 15th day. This would be in accordance with expectation on the basis of the data given in Fig. 10 of a previous paper (Vallance, 1950 *a*) and is substantiated by the work relating to respiration and germination of immature seeds.

The data of the 18- to 30-day period of the experiment illustrated by Fig. 7*a* deserve some mention, but since no comparable data have been obtained from experiments on seed of other years, the interpretation offered is largely speculative. It will be seen that from 24 to 30 days the rate of gaseous exchange diminished and at the same time the R.Q. dropped from 0·79 to 0·47.

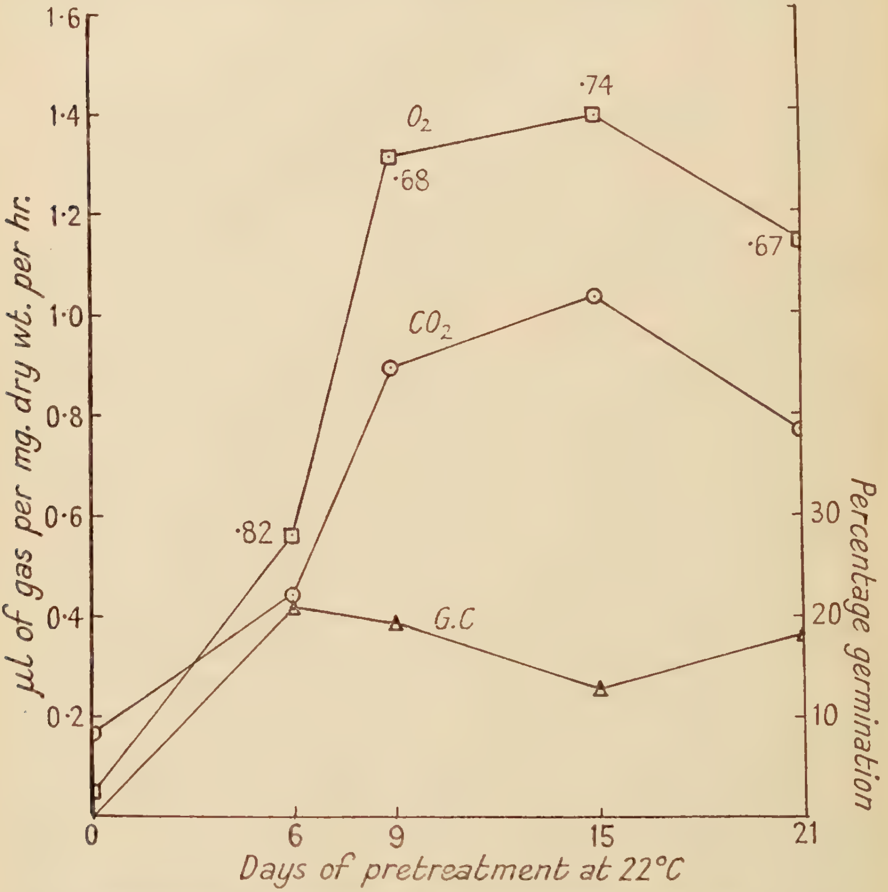

Fig. 8. The relationship between length of pretreatment at 22° C. on the respiration and germination capacity (G.C.) of overripe 1941 seed. (Standard error of respiratory data = ±0·040.)

Presumably, therefore, after 30 days of pretreatment respiration was almost completely protein in nature. Consequently it seems possible that the fall in the germination between 27 and 30 days was due to the exhaustion of the specific metabolite as a result of its utilization as a respiratory substrate.

A further batch of the 1941 seed was received in December 1947, and, with a view to limited chemical analysis of these seeds throughout pretreatment, further experiments on germination and the drift of respiration were carried out. Unfortunately the new batch of seed was overripe on arrival and the results obtained were so different from those shown in Fig. 7 *a* that the original intention was not fulfilled. Nevertheless these results showed some features which may be of interest and hence they are included in the present paper. Fig. 8 illustrates some of the data obtained from the experiments on overripe seed. It will be noticed in this figure that whereas the level of respiration at the 6th day was lower than at the comparable time in Fig. 7 *a*, at the 9th, 15th, and 21st days respectively it was much higher. The R.Q.s at these later times were 0·68, 0·74, and 0·67 respectively, whereas at the same times in Fig. 7 *a* the R.Q.s were 0·82, 1·00, and 0·91. Furthermore, the maximum germination capacity of the overripe seed at the time of the experiment was 20·6 per cent., whereas when the seed was at the stage at which it was considered to be ripe it was 63·0 per cent. In view of the higher level of respiration of the overripe seed compared with that of the seed at the stage at which it was only ripe, the germination data were in accordance with expectation.

However, the most striking difference between the overripe seed and immature and ripe seed was with respect to the effect of the stimulating solution on seed respiration. This may be seen from Fig. 9, which shows the effect of the stimulating solution on the respiration of overripe seeds during the first 9 hours after exposure to the stimulating solution. The seeds used had been pretreated at 22° C. for 6, 9, 15, and 21 days respectively before being placed in the stimulating solution. It will be seen from Fig. 9 that after 6 days of pretreatment the effect of the stimulating solution was as expected from previous data on other seeds (Vallance, 1950 *b*), since the peak output of carbon dioxide was 1·24 μl. per mg. dry weight per hour. In contrast to this, after 9, 15, and 21 days of pretreatment the peak output of carbon dioxide was 1·76 μl., 2·18 μl. and 2·02 μl. respectively. Moreover, the germination capacities of the seeds after 6, 9, and 21 days did not differ significantly. Clearly then, even though it was impossible to distinguish between the respiration of those seeds which would germinate at these times and those which would not, the high levels of respiration attained could not have been due only to the germinable seeds. It might, however, be argued that the increases in the level of respiration recorded were only apparent since, throughout pretreatment, the seeds must have lost appreciable amounts of carbon in the form of carbon dioxide. Calculation showed that the total loss of carbon up to the 21st day of pretreatment could not have been more than 20 per cent. of the initial dry weight of the seeds, and in consequence the differences between the peak rate of respiration at 6 days and at the other times must have been real and not apparent. Possibly there is some parallel between the respiratory phenomena shown by senescent fruits and that of overripe seeds of *Striga hermonthica*, in which in any case germination capacity is falling off.

DISCUSSION

The data given show beyond all reasonable doubt that the germination capacity of the seeds of *Striga hermonthica* and their respiration during

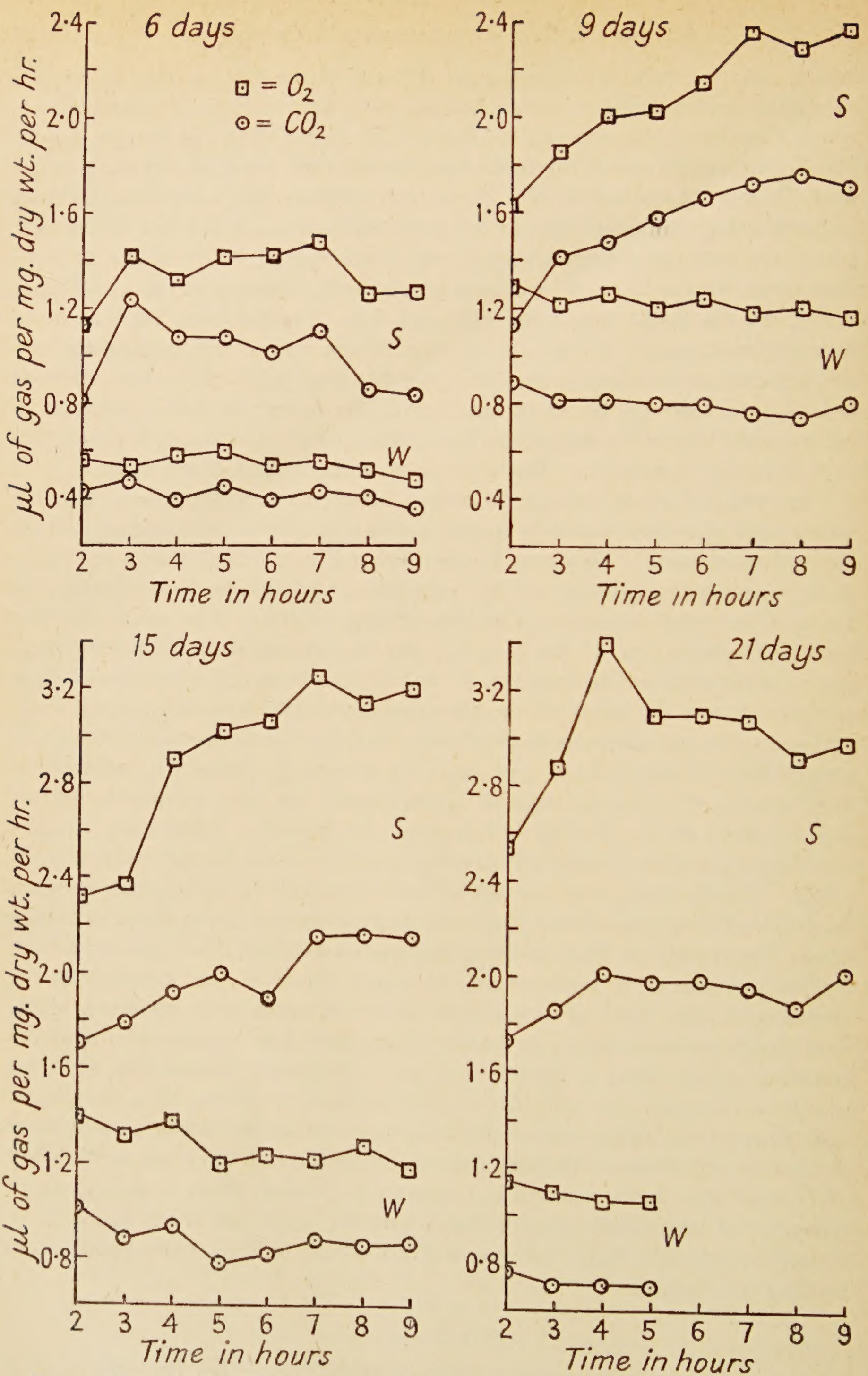

FIG. 9. The effect of the stimulating solution on the respiration of overripe seeds after periods of pretreatment of 6, 9, 15, and 21 days respectively. ((S) respiration in stimulating solution; (W) respiration in distilled water.)

pretreatment are linked in some way. Thus when the respiration of the seeds exceeds a certain critical value during the first phase of pretreatment, the seeds are unable to germinate however much pretreatment is prolonged. Furthermore, the peak level of the respiration which can be attained by the seeds during pretreatment falls as the seed after-ripens, and hence the seeds are able to germinate progressively as after-ripening proceeds. In view of this it would seem that as the seed after-ripens, certain changes take place which could be interpreted as having the effect of restricting gaseous diffusion when the seeds are exposed to moisture. Initially it was considered that the testa might form the requisite barrier to gaseous diffusion, but this now seems unlikely, for in the case of those seeds in which dormancy has been shown to be due to the testa, it has been found (Barton, 1947) that the permeability of the testa increases as dry storage is prolonged. The exact nature of the barrier in the present instance has not been established, but it is now thought possible that the single layer of perisperm might be able to act as a differentially permeable layer.

In the latter stages of after-ripening it would seem that some factor other than permeability is involved, for in spite of a comparatively high level of respiration optimum germination takes place. Nevertheless, when the seed becomes over-ripe its peak rate of respiration during moisture-treatment is higher than that of the ripe seed over certain comparable periods. Consequently the germination capacity of the seed diminishes when after-ripening is prolonged beyond a certain limit.

The fall in the rate of gaseous exchange during pretreatment (shown in Figs. 1, 4, and 6) has been ascribed to carbon dioxide narcosis, largely by analogy with the results achieved in the experiments of Kidd (1914, 1915) and Kidd and West (1917). If this hypothesis is correct, then it would again seem that some barrier capable of restricting gaseous diffusion must be present in the seed. Once again it would seem that the only layer of the seed which is capable of fulfilling this function is the perisperm.

Full elucidation of the changes which take place inside the seeds during pretreatment and which enable the seed to germinate in the presence of the stimulating solution has not yet been possible. However, experiments on the effect of the stimulating solution on seed respiration have shown that, at times when the seeds are able to germinate, the respiratory substrate utilized is different from that which is used at times when the seeds are unable to germinate. As a result of these experiments it is thought that germination may depend on the accumulation of some specific metabolite during pretreatment. The rate of mobilization of this metabolite would appear to be greater in ripe seed than in unripe seed. On this hypothesis, if germination is to take place, then sufficient of the metabolite must be available to enable some specific reaction, with which the stimulant may or may not be directly involved, to proceed at a certain rate. Furthermore, if the theory advanced is correct, then independent germination would be due in part to an accumulation of the metabolite sufficient to enable the reaction to proceed autocatalytically.

Now the stimulant has been shown to enhance seed respiration at all stages of pretreatment, even at the stage when the seeds may possibly be influenced by carbon dioxide narcosis. Furthermore, experiments with 1948 and 1949 seeds have shown that when very immature dry seed is added both to the stimulating solution and to distilled water, the peak respiration attained in each case is eventually much the same. The data of these and other experiments suggest that the active substance of the stimulating solution is not a specific respiratory enzyme, but rather that one of its main effects is to increase the permeability of some part of the seed to gaseous exchange. Now it is known (Brown, Robinson, and Johnson, 1949) that the stimulating solution induces the germination of pretreated seeds by promoting extension growth of the radicle. Whether or not the possible effect of the stimulating solution on permeability is in itself sufficient to account for the promotion of radicle growth is still not clear.

LITERATURE CITED

ANDREWS, F. W., 1945: The Parasitism of *Striga hermonthica* Benth. on Sorghum spp. Under Irrigation. Ann. Appl. Biol., xxxii. 193.

BARTON, L. V., 1947: Special Studies on Seed Coat Impermeability. C.B.T.I., xiv. 355.

BROWN, R., and EDWARDS, M., 1944: The Germination of the Seed of *Striga lutea*. I. Ann. Bot., viii. 131.

—— —— 1946: The Germination of the Seeds of *Striga lutea*. II. Ibid., x. 133.

—— ROBINSON, E., and JOHNSON, A. W., 1949: Effect of the *Striga* Germination Stimulant on Extension Growth on the Roots of Peas. Nature, cliii. 842.

CHABROLIN, C., 1938: La Germination des graines de l'*Orobanche speciosa*. C. R. d. l'Acad. des Sci., ccvi. 1990.

KIDD, F., 1914: The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds. Part I. Proc. Roy. Soc., B, lxxxvii. 408.

—— 1914: The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds. Part II. Ibid., 609.

—— 1915: The Controlling Influence of Carbon Dioxide. Part III. Ibid., B, lxxxix. 136.

—— and WEST, C., 1917: The Controlling Influence of Carbon Dioxide. IV. Ann. Bot., xxxi. 457.

SAUNDERS, A. R., 1933: Studies in Phanerogamic Parasitism with Particular Reference to *Striga lutea*. Lour. Dept. Agric., Union of S. A., Sci., Bull. No. 128.

VALLANCE, K. B., 1950 *a*: Studies on the Germination of the Seeds of *Striga hermonthica*. I. Ann. Bot., xiv. 347.

—— 1950 *b*: Studies on the Germination of the Seeds of *Striga hermonthica*. II. J. Exp. Bot. (in press).

WEINMANN, H., 1944: Semi-micro Estimation of Reducing Sugars. Plant Physiol., xix. 148.

—— 1947: Determination of Total Available Carbohydrates in Plants. Ibid., xxii. 279.